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(54) Title: TREATMENT TO PREVENT LOSS OF AND/OR INCREASE BONE MASS IN METABOLIC BONE DISEASES														
(57) Abstract	The invention is a treatment for increasing the bone mass or preventing bone loss in an individual afflicted with a bone disease which includes administering to the individual a morphogen in a therapeutically effective amount so as to maintain or stimulate bone formation.													

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TREATMENT TO PREVENT LOSS OF
AND/OR INCREASE BONE MASS IN METABOLIC BONE DISEASES

- 5 This invention relates to means for increasing the bone mass and/or preventing the loss of bone mass in a mammal.

Background of the Invention

10

Throughout adult life, bone is continually undergoing remodeling through the interactive cycles of bone formation and resorption (bone turnover). Bone resorption typically is rapid, and is mediated by 15 osteoclasts (bone resorbing cells), formed by mononuclear phagocytic precursor cells at bone remodeling sites. This process then is followed by the appearance of osteoblasts (bone forming cells) which form bone slowly to replace the lost bone. The 20 activities of the various cell types that participate in the remodeling process are controlled by interacting systemic (e.g., hormones, lymphokines, growth factors, vitamins) and local factors (e.g., cytokines, adhesion molecules, lymphokines and growth factors). The fact 25 that completion of this process normally leads to

balanced replacement and renewal of bone indicates that the molecular signals and events that influence bone remodeling are tightly controlled.

- 5 A number of bone growth disorders are known which cause an imbalance in the bone remodeling cycle. Chief among these are metabolic bone diseases, such as osteoporosis, osteoplasia (osteomalacia), chronic renal failure and hyperparathyroidism, which result in
- 10 abnormal or excessive loss of bone mass (osteopenia). Other bone diseases, such as Paget's disease, also cause excessive loss of bone mass at localized sites.

Osteoporosis is a structural deterioration of the skeleton caused by loss of bone mass resulting from an imbalance in bone formation, bone resorption, or both, such that the resorption dominates the bone formation phase, thereby reducing the weight-bearing capacity of the affected bone. In a healthy adult, the rate at which bone is formed and resorbed is tightly coordinated so as to maintain the renewal of skeletal bone. However, in osteoporotic individuals an imbalance in these bone remodeling cycles develops which results in both loss of bone mass and in formation of microarchitectural defects in the continuity of the skeleton. These skeletal defects, created by perturbation in the remodeling sequence, accumulate and finally reach a point at which the structural integrity of the skeleton is severely compromised and bone fracture is likely. Although this imbalance occurs gradually in most individuals as they

age ("senile osteoporosis"), it is much more severe and occurs at a rapid rate in postmenopausal women. In addition, osteoporosis also may result from nutritional and endocrine imbalances, hereditary disorders and a

- 5 number of malignant transformations.

Patients suffering from chronic renal (kidney) failure almost universally suffer loss of skeletal bone mass (renal osteodystrophy). While it is known that

- 10 kidney malfunction causes a calcium and phosphate imbalance in the blood, to date replenishment of calcium and phosphate by dialysis does not significantly inhibit osteodystrophy in patients suffering from chronic renal failure. In adults, 15 osteodystrophic symptoms often are a significant cause of morbidity. In children, renal failure often results in a failure to grow, due to the failure to maintain and/or to increase bone mass.

- 20 Osteoplasia, also known as osteomalacia ("soft bones"), is a defect in bone mineralization (e.g., incomplete mineralization), and classically is related to vitamin D deficiency (1,25-dihydroxy vitamin D₃). The defect can cause compression fractures in bone, and 25 a decrease in bone mass, as well as extended zones of hypertrophy and proliferative cartilage in place of bone tissue. The deficiency may result from a nutritional deficiency (e.g., rickets in children), malabsorption of vitamin D or calcium, and/or impaired 30 metabolism of the vitamin.

- Hyperparathyroidism (overproduction of the parathyroid hormone) is known to cause malabsorption of calcium, leading to abnormal bone loss. In children, hyperparathyroidism can inhibit growth, in adults the skeleton integrity is compromised and fracture of the ribs and vertebrae are characteristic. The parathyroid hormone imbalance typically may result from thyroid adenomas or gland hyperplasia, or may result from prolonged pharmacological use of a steroid. Secondary hyperparathyroidism also may result from renal osteodystrophy. In the early stages of the disease osteoclasts are stimulated to resorb bone in response to the excess hormone present. As the disease progresses, the trabecular bone ultimately is resorbed and marrow is replaced with fibrosis, macrophages and areas of hemorrhage as a consequence of microfractures. This condition is referred to clinically as osteitis fibrosa.
- 20 Paget's disease (osteitis deformans) is a disorder currently thought to have a viral etiology and is characterized by excessive bone resorption at localized sites which flare and heal but which ultimately are chronic and progressive, and may lead to malignant transformation. The disease typically affects adults over the age of 25.

To date, osteopenia treatments are based on inhibiting further bone resorption, e.g., by 1) inhibiting the differentiation of hemopoietic mononuclear cells into mature osteoclasts, 2) by directly preventing osteoclast-mediated bone resorption, or 3) by affecting the hormonal control of bone resorption. Drug regimens used for the treatment of osteoporosis include calcium supplements, estrogen,

calcitonin and diphosphonates. Vitamin D₃ and its metabolites, known to enhance calcium and phosphate absorption, also are being tried. None of the current therapies stimulate regeneration of new bone tissue.

- 5 In addition, all of these agents have only a transient effect on bone remodeling. Thus, while in some cases the progression of the disease may be halted or slowed, patients with significant bone deterioration remain actively at risk. This is particularly prevalent in
- 10 disorders such as osteoporosis where early diagnosis is difficult and/or rare and significant structural deterioration of the bone already may have occurred.

It is an object of the present invention to develop

- 15 methods and compositions for inhibiting or preventing the loss of bone mass and/or for increasing bone formation in an individual who, for example, is afflicted with a disease which decreases skeletal bone mass, particularly where the disease causes an
- 20 imbalance in bone remodeling. Another object is to enhance bone growth in children suffering from bone disorders, including metabolic bone diseases. Still another object is to prevent or inhibit bone deterioration in individuals at risk for loss of bone
- 25 mass, including postmenopausal women, aged individuals, and patients undergoing dialysis. Yet another object is to provide methods and compositions for repairing defects in the microstructure of structurally compromised bone, including repairing bone fractures.
- 30 Thus, the invention is aimed at stimulating bone

- 6 -

formation and increasing bone mass, optionally over prolonged periods of time, and particularly to decrease the occurrence of new fractures resulting from structural deterioration of the skeleton. These and 5 other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

The present invention provides methods and compositions for inhibiting loss of bone mass, and/or
5 for stimulating bone formation in mammals, particularly humans.

In one aspect, the invention features a therapeutic treatment method and composition for preventing loss of
10 bone mass and/or for increasing bone mass in a mammal which includes administering to the individual a therapeutically effective morphogen in an amount and for a time sufficient to inhibit the loss of bone mass, and/or to increase bone mass in the individual.
15

In another aspect, the invention features a therapeutic treatment method and composition for preventing loss of bone mass and/or for increasing bone mass in a mammal which includes administering to the
20 mammal a compound that stimulates in vivo a therapeutically effective concentration of an endogenous morphogen in the body of the mammal sufficient to prevent loss of and/or to increase bone mass in the individual. These compounds are referred
25 to herein as morphogen-stimulating agents, and are understood to include substances which, when administered to a mammal, act on tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen, and
30 which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or secretion of an endogenous morphogen.

The morphogens described herein are believed to play a significant role in maintaining appropriate bone mass in an individual. Thus, a morphogen may be administered according to the invention to any 5 individual who requires assistance in maintaining appropriate bone mass and/or who suffers from a bone remodeling imbalance. For example, the morphogen or morphogen-stimulating agent may be administered according to the invention to an adult suffering from 10 renal failure to prevent bone deterioration which is associated with that disease, e.g., to correct bone loss due to late stage kidney failure. Similarly, the administration of a morphogen to a child suffering from renal failure is expected not only to alleviate loss of 15 bone mass in the child, but also to stimulate bone formation and thus growth. In addition, administration of a morphogen or morphogen-stimulating agent to an individual suffering from defects in skeletal microstructure is expected to result in repair of that 20 defect, and to enhance the weight-bearing capacity of the treated bone.

Accordingly, in another aspect of the invention, the treatment methods and compositions of the invention 25 may be used to treat a bone fracture or any disease which causes or results in bone fractures or other defects in skeletal microstructure, including loss of bone mass, and which compromise the weight-bearing capacity of bone. Such diseases include, for example, 30 chronic renal failure and other kidney diseases, particularly those requiring dialysis; osteomalacia; vitamin D deficiency-induced osteopenia or osteoporosis; postmenopausal or senile osteoporosis; hyperparathyroidism and Paget's disease.

- In still another aspect, the invention provides methods and compositions for protecting an individual at risk for the loss or deterioration of skeletal bone mass by prophylactic administration of a morphogen or 5 morphogen-stimulating agent. Individuals at risk include postmenopausal females, aged individuals, and individuals undergoing dialysis, particularly prolonged or chronic dialysis.
- 10 In one preferred embodiment of the invention, the morphogen or morphogen-stimulating agent is administered systemically to the individual, e.g., orally or parenterally. In another embodiment of the invention, the morphogen may be provided directly to 15 the bone, e.g., by injection to the bone periosteum or endosteum. Direct injection is particularly useful for repairing defects in the microstructure of the bone, including bone fractures.
- 20 In any treatment method of the invention, "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the mature form of the morphogen may be provided in 25 association with its precursor "pro" domain, which is known to enhance the solubility of the protein. Other useful molecules known to enhance protein solubility include casein and other milk components, as well as various serum proteins. Additional useful molecules 30 which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-stimulating agent to bone. Tissue targeting molecules envisioned to be useful in the treatment 35 protocols of this invention include tetracycline,

diphosphonates, and antibodies or other binding proteins which interact specifically with surface molecules on bone tissue cells.

5 Still another useful tissue targeting molecule is the morphogen precursor "pro" domain, particularly that of OP-1, BMP2 or BMP4. These proteins are found naturally associated with bone tissue but likely are synthesized in other tissues and targeted to bone
10 tissue after secretion from the synthesizing tissue. For example, the primary source of OP-1 synthesis appears to be the tissue of the urinary tract (e.g., renal tissue), while the protein has been shown to be active in bone tissue (see below.) Moreover, the
15 protein has been identified in serum, saliva and various milk forms. In addition, the secreted form of the protein comprises the mature dimer in association with the pro domain of the intact morphogen sequence. Accordingly, the associated morphogen pro domains may
20 act to target specific morphogens to different tissues in vivo.

Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the
25 morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of bone remodeling sites.

30 The morphogens or morphogen-stimulating agents also may be administered together with other "co-factors" known to have a beneficial effect on bone remodeling, including parathyroid hormone, vitamin D₃, prostaglandins, dexamethasone, IGF (I, II) and their

binding proteins, and other agents known to enhance osteoblast activity. Other useful confactors include calcitonin and estrogen and other agents which inhibit bone resorption.

5

- Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from 10 Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), and the recently identified 60A protein (from Drosophila, Seq. 15 ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a 20 precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be 25 predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication 30 sources for the amino acid sequences of the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

5 "OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

10 "OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

15 "OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

20 "OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

25 "OP-2" Refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

30 "OP-2" Refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

35 "OP-2" Refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.). The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.). The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues further upstream for both OP-2 proteins.)

"CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature collectively as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

- 5 "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.
- 10
- 15 "Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The pro domain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.
- 20
- 25 "Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The pro domain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.
- 30

- 5 "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The pro domain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.
- 10
- 15 "60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The pro domain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.
- 20
- 25
- 30 "BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
- 35

"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appear sin Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in

combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the 5 C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not 10 their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain 15 disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of 20 progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, 25 it also is anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of 30 this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. 35 Generic Sequence 1 comprises the conserved six cysteine

skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further 5 comprise the following additional sequence at their N- terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

1 5

10

Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic 15 Sequence 6 (Seq. ID No. 31), listed below. These Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically, 20 Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 25 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both 30 the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that

these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 3

Leu Tyr Val Xaa Phe

10	1	5
	Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa	
	10	
	Xaa Ala Pro Xaa Gly Xaa Xaa Ala	
15	15	20
	Xaa Tyr Cys Xaa Gly Xaa Cys Xaa	
	25	30
	Xaa Pro Xaa Xaa Xaa Xaa Xaa	
	35	
	Xaa Xaa Xaa Asn His Ala Xaa Xaa	
20	40	45
	Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa	
	50	
	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys	
	55	60
25	Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa	
	65	

Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Gly Cys Xaa
95

wherein each Xaa is independently selected from a group
10 of one or more specified amino acids defined as
follows: "Res." means "residue" and Xaa at res.4 =
(Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or
Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu
or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn);
15 Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile
or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =
(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr
or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu
or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at
20 res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
(Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro
or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala
or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala);
25 Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn
or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at
res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or
Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 =
30 (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
res.49 = (Val or Met); Xaa at res.50 = (His or Asn);
Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa

at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at 5 res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at 10 res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = 15 (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His); 20 Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or Arg);

25

Generic Sequence 4

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe
	1				5				10	
30	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
					15					
	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala		
	20					25				
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
35				30			35			

Xaa Pro Xaa Xaa Xaa Xaa Xaa
40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
45 50
5 Xaa Xaa Leu Xaa Xaa Xaa Xaa
55
Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Pro Xaa Xaa Xaa Xaa
10 70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
15 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Gly Cys Xaa
100

wherein each Xaa is independently selected from a group
20 of one or more specified amino acids as defined by the
following: "Res." means "residue" and Xaa at res.2 =
(Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4
= (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg
or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at
25 res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp
or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 =
(Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg,
or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 =
(Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro
30 or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 =
(Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 =
(Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp
or Gln); Xaa at res.33 = (Glu, Lys, Asp or Gln); Xaa at
res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu
35 or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 =

(Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, 5 Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = 10 (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = 15 (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, 20 Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, 25 Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically,

5 Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP

10 (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14 and 32), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60A (from Drosophila, Seq. ID No. 24). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the

15 variable positions within the sequence. As for Generic Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or

20 intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the

25 tertiary structure of the proteins.

Generic Sequence 5

30

Leu Xaa Xaa Xaa Phe

1

5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

10

Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
15 20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30

5 Xaa Pro Xaa Xaa Xaa Xaa Xaa
35

Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
10 50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65

15 Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80

Xaa Xaa Xaa Xaa Met Xaa Val Xaa
20 85 90

Xaa Cys Xaa Cys Xaa
95

wherein each Xaa is independently selected from a group
of one or more specified amino acids defined as
25 follows: "Res." means "residue" and Xaa at res.2 =
(Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4
= (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8

= (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18
5 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu, 10 Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or 15 Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = 20 (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at 25 res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or 30 Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at 35 res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met

or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 =
 5 (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile);
 10 Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

15 Generic Sequence 6

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Phe
	1					5			10
	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa
20						15			
	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Ala	
	20					25			
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa	
			30					35	
25	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
						40			
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa	
			45					50	
	Xaa								
30						55			
	Xaa	Cys							
		60					65		
	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	
						70			

- Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Xaa Cys Xaa
100
- 10 wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 = (Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln, Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.33 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.35 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at res.39 = (Asn, Asp, Ala; Thr or Pro); Xaa at res.40 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,

Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
5 res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
10 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
15 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or
Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at
res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or
20 Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,
Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or
Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa
at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile,
25 Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at
res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =
(Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro);
Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 =
(Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,
30 Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser);
Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =
(His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see 5 Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Table II, below, and Seq. ID Nos. 24-28), all of which include at least the conserved six or seven cysteine skeleton. In addition, 10 biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). 15 Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic and species variants and mutants, 20 and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences 25 include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979). As used herein, potentially useful sequences are aligned with a 30 known morphogen sequence using the method of Needleman et al. ((1970) J.Mol.Biol. 48:443-453) and identities calculated by the Align program (DNAsstar, Inc.). "Homology" or "similarity" as used herein includes allowed conservative changes as defined by Dayoff et 35 al.

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence

5 defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another

10 preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various

15 identified species of OPI and OP2 (Seq. ID No. 29).

The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains

20 described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various

25 truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these

30 cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of

35 related proteins having regions of amino acid sequence

homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

5 The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently
10 preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods and compositions of this invention is disclosed in copending US patent application Serial Nos. 752,764,
15 filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosures of which are incorporated herein by reference.

Thus, in view of this disclosure, skilled
20 genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both
25 procaryotes and eucaryotes, to produce large quantities of active proteins capable of enhancing bone formation and/or inhibiting abnormal bone deterioration in a variety of mammals, including humans, for use in maintaining appropriate bone mass and bone remodeling
30 in developing and adult bone tissue.

Brief Description of the Drawings

The foregoing and other objects and features of this invention, as well as the invention itself, may be 5 more fully understood from the following description, when read together with the accompanying drawings, in which:

FIG. 1 compares the mitogenic effect of hOP-1 and 10 TGF- β on rat osteoblasts;

FIG. 2 illustrates the effect of human osteogenic protein-1 (hOP-1) on the collagen synthesis of osteoblasts;

15 FIG. 3 compares the alkaline phosphatase induction effect of hOP-1 and TGF- β on rat osteoblasts;

20 FIG. 4 shows the long-term effect of hOP-1 on the production of alkaline phosphatase by rat osteoblasts;

25 FIG. 5 shows the effect of hOP-1 on parathyroid hormone-mediated cAMP production using rat osteoblasts in culture;

FIG. 6A and B graphs the effect of morphogen on osteoclastin synthesis (A), and the effect of morphogen on the rate of mineralization (B);

30 FIG. 7 shows Western Blot analysis of bovine colostrum using OP-1 and BMP2-specific antibodies;

FIG. 8A and B show results of in vivo and in vitro activity assays, respectively, for mammary extract purified OP-1;

5 FIG. 9 is a photomicrograph of an immunoblot showing the presence of hOP-1 in serum; and

10 FIG. 10 (A and B) are photomicrographs showing new endosteum bone formation following morphogen injection onto the endosteal surface (A), and new periosteum bone formation following morphogen injection onto the periosteal surface (B);

15 FIG. 11 is a graphic representation of the dose-dependent effect of morphogen on bone resorption; and

FIG. 12 (A and B) are schematic representations of morphogen inhibition of early mononuclear phagocytic cell multinuclearization in vivo;

Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for preventing
5 loss of bone mass and/or for stimulating bone formation when provided systemically or injected directed into bone tissue in a mammal. As described herein, these proteins ("morphogens") may be used in the treatment of metabolic bone diseases and other disorders that cause
10 an imbalance of the bone remodeling cycle, and/or which cause deterioration of the skeletal microstructure.

The invention is based on the discovery of a family of morphogenic proteins capable of inducing tissue
15 morphogenesis in a mammal. More particularly, the invention is based on the discovery that these proteins play an important role, not only in embryogenesis, but also in the growth, maintenance and repair of bone tissue in juvenile and adult mammals.

20 It has been shown that implantation of a morphogen (including OP-1, CBMP2, DPP and 60A protein, and various biosynthetic constructs, such as COP5 and COP7) together with a suitable matrix in subcutaneous sites
25 in mammals induces a sequence of cellular events which leads to the formation of fully functional new bone, as determined by the specific activity of alkaline phosphatase, calcium content and histology of day 12 implants (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691, and USSN 667,274 and 752,857, the disclosures of which are incorporated herein by reference.) The morphogen-containing implants recruit nearby mesenchymal stem cells and trigger their differentiation into chondrocytes within 5-7 days.
30 Upon capillary invasion, the chondrocytes hypertrophy,
35

become calcified and subsequently are replaced by newly formed bone within 9-12 days. The mineralized bone then is remodeled extensively and becomes occupied by ossicles filled with functional bone marrow elements by
5 14-21 days.

As described herein, the morphogens provided herein stimulate the proliferation, growth and differentiation of osteoblasts in vitro (see Examples 2-7, below), and
10 can induce bone formation in osteoporotic bone tissue in vivo when provided systemically to a mammal, or directly to bone tissue, without an associated matrix carrier (see Examples 8, 9, below.) In addition, the morphogens inhibit multinucleation of activated early
15 mononuclear phagocytic cells (see Example 12, below). Moreover, inhibition of endogenous morphogen activity can inhibit normal skeleton development in a mammal (see Example 13, below.)

20 As described in Example 1 and in detail in copending USSN 752,764 and 752,861, the disclosures of which are incorporated herein by reference, the naturally-occurring morphogens are widely distributed in the different tissues of the body. For example, as
25 determined by northern blot hybridization, OP-1 is expressed primarily in the tissue of the urogenital tract (e.g., renal and bladder tissues). By contrast, Vgr-1, BMP3, BMP4 and BMP5 appear to be expressed primarily in the heart and lung. BMP5 also appears to
30 be expressed significantly in liver tissue. GDF-1 appears to be expressed primarily in brain tissue. (See, for example, Ozkaynak et al. (1992) JBC, in publication.) Moreover, the tissue of synthesis may differ from the natural site of action of specific
35 morphogens. For example, although OP-1 appears to be

primarily synthesized in renal tissue, the protein is active in bone tissue. In addition, at least one morphogen, OP-1, is present in a number of body fluids, including saliva, milk (including mammary gland extract, colostrum and 57-day milk) and serum (see Example 11, below.) Accordingly, without being limited to a given theory, the morphogens described herein may behave as endocrine factors, e.g., proteins secreted from a factor-producing tissue in response to particular stimuli, and capable of being transported to, and acting on, a distant tissue. These findings further distinguish morphogens from other members of the TGF- β superfamily of proteins, including TGF- β , which act as local or autocrine factors produced by the tissue on which they act.

The pro domain may function to enhance protein solubility and/or to assist in tissue targeting of morphogens to particular tissues. For example, the mature, active form of OP-1 appears to be secreted from cells in association with the pro domain of the intact sequence. Accordingly, while, as explained herein, the morphogens useful in this invention have significant amino acid sequence homologies within the active domains and are similar in their ability to induce tissue morphogenesis, without being limited to any theory, it is hypothesized that the sequence variation within the morphogenic protein family members may reflect the different specific roles each morphogen plays in specific tissues under natural occurring conditions. For example, the significant sequence variation within the pro domains may mean that these regions of the protein sequence are important for targeting specific morphogens to different tissues for morphogenic activity therein.

Accordingly, the present invention comprises two fundamental aspects. In one aspect, the methods and compositions of this invention comprise a morphogen which, when administered to an individual, is capable 5 of inhibiting loss of bone mass and/or stimulating bone formation in the individual. In another aspect, the methods and compositions of the invention comprise a morphogen-stimulating agent which, when administered to an individual, is capable of inducing the expression 10 and/or secretion of sufficient endogenous morphogen within the individual to provide therapeutically effective concentrations capable of inhibiting loss of bone mass and/or stimulating bone formation in the individual.

15

Example 14 describes an assay for screening compounds to identify candidate morphogen-stimulating agents. A detailed description of useful screening assays for identifying candidate morphogen-stimulating 20 agents also is provided in USSN 752,861, the disclosure of which is incorporated herein by reference. Candidate agents then may be tested for their efficacy in vivo using, for example, the osteoporosis model described in Examples 8 and 9 below.

25

Provided below are detailed descriptions of suitable morphogens useful in the methods and compositions of this invention, as well as methods for the administration and application of these morphogens 30 and/or of morphogen-stimulating agents. Also provided are numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-

stimulating agents described herein as therapeutic agents for inhibiting abnormal bone loss and/or for enhancing bone formation in a human, and 2) provide assays with which to test candidate morphogens and 5 morphogen-stimulating agents for their efficacy.

I. Useful Morphogens

10 As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton 15 or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the 20 differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogens useful in the method of 25 this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30, 1991, the disclosures of which are hereby incorporated 30 by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from prokaryotic or eucaryotic

host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

5

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat.

10 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

Accordingly, the morphogens useful in the methods
15 and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences described above, where "homology" is as defined herein
20 above.

The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3,
25 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

1

5

30

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1
35 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2

(Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from *Drosophila*, Seq. ID No. 11), Vgl, (from *Xenopus*, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), 5 GDF-1 (from mouse, Seq. ID No. 14, 32 and 33), 60A protein (from *Drosophila*, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method of Needleman et al. (1970) *J. Mol. Biol.*, 48:443-453, 10 calculated using the Align Program (DNASTar, Inc.). In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of 15 illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser 20 and Ile.

TABLE II

25

	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
	mOP-1
	hOP-2	...	Arg	Arg
	mOP-2	...	Arg	Arg
30	DPP	...	Arg	Arg	...	Ser
	Vgl	Lys	Arg	His
	Vgr-1	Gly
	CBMP-2A	Arg	...	Pro
	CBMP-2B	...	Arg	Arg	...	Ser
35	BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...

GDF-1	...	Arg	Ala	Arg	Arg
60A	...	Gln	Met	Glu	Thr
BMP5
BMP6	...	Arg
5		1			5			

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1
10 hOP-2	Gln	Leu	...
mOP-2	Ser	Leu	...
DPP	Asp	...	Ser	...	Val	Asp	...
Vgl	Glu	...	Lys	...	Val	Asn
Vgr-1	Gln	...	Val
15 CBMP-2A	Asp	...	Ser	...	Val	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	Asn	...
BMP3	Asp	...	Ala	...	Ile	Ser	Glu
GDF-1	Glu	Val	His	Arg
60A	Asp	...	Lys	His	...
20 BMP5
BMP6	Gln
		10					15		

hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
25 mOP-1
hOP-2	...	Val	Gln	Ser
mOP-2	...	Val	Gln	Ser
DPP	Val	Leu	Asp
Vgl	...	Val	Gln	Met
30 Vgr-1	Lys
CBMP-2A	Val	Pro	His
CBMP-2B	Val	Pro	Gln
BMP3	Ser	...	Lys	Ser	Phe	Asp

GDF-1	...	Val	Arg	...	Phe	Leu
60A	Gly
BMP5
BMP6	Lys
5				20					25

hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1
10 hOP-2	Ser
mOP-2
DPP	His	...	Lys	...	Pro
Vgl	...	Asn	Tyr	Pro
Vgr-1	...	Asn	Asp	Ser
15 CBMP-2A	...	Phe	His	...	Glu	...	Pro
CBMP-2B	...	Phe	His	...	Asp	...	Pro
BMP3	Ser	...	Ala	...	Gln
GDF-1	...	Asn	Gln	...	Gln
60A	...	Phe	Ser	Asn
20 BMP5	...	Phe	Asp	Ser
BMP6	...	Asn	Asp	Ser
				30					35

hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
25 mOP-1
hOP-2	Asp	...	Cys
mOP-2	Asp	...	Cys
DPP	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	Thr	Glu	Ile	Leu	...	Gly
30 Vgr-1	Ala	His
CBMP-2A	Ala	Asp	His	Leu	...	Ser
CBMP-2B	Ala	Asp	His	Leu	...	Ser
GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...

BMP3	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	Ala	His
BMP5	Ala	His	Met
BMP6	Ala	His	Met

5

40

hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP-1
hOP-2	Leu	...	Ser	...
10 mOP-2	Leu	...	Ser	...
DPP	Val
Vgl	Ser	Leu
Vgr-1
CBMP-2A
15 CBMP-2B
BMP3	Ser	Thr	Ile	...	Ser	Ile
GDF-1	Leu	Val	Leu	Arg	Ala	...
60A
BMP5
20 BMP6

45

50

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
25 mOP-1	Asp
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
DPP	...	Asn	Asn	Asn	Gly	Lys	...
Vgl	Ser	...	Glu	Asp	Ile
30 Vgr-1	Val	Met	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
BMP3	...	Arg	Ala** Gly	Val	Val	Pro	Gly	Ile	

	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
	60A	Leu	Leu	Glu	...	Lys	Lys	...
	BMP5	Leu	Met	Phe	...	Asp	His	...
	BMP6	Leu	Met	Tyr	...
5			55					60		
	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	mOP-1
10	hOP-2	Ala	Lys
	mOP-2	Ala	Lys
	DPP	Ala	Val
	Vgl	...	Leu	Val	Lys
	Vgr-1	Lys
15	CBMP-2A	Ala	Val	Glu
	CBMP-2B	Ala	Val	Glu
	BMP3	...	Glu	Val	...	Glu	Lys
	GDF-1	Asp	Leu	Val	...	Ala	Arg
	60A	Arg
20	BMP5	Lys
	BMP6	Lys
			65					70		
	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
25	mOP-1
	hOP-2	...	Ser	...	Thr	Tyr
	mOP-2	...	Ser	...	Thr	Tyr
	Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
	Vgr-1	Val
30	DPP	...	Asp	Ser	Val	Ala	Met	Leu
	CBMP-2A	...	Ser	Met	Leu
	CBMP-2B	...	Ser	Met	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr

	GDF-1	...	Ser	Pro	The	...
	60A	...	Gly	...	Leu	Pro	His
	BMP5
	BMP6
5					75					80

	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
10	mOP-2	...	Ser	...	Asn	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val
	Vgl	...	Asn	Asn	Asp	Val	...	Arg
	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
15	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
	BMP3	...	Glu	Asn	Lys	Val
	GDF-1	...	Asn	...	Asp	Val	...	Arg
	60A	Leu	Asn	Asp	Glu	Asn
	BMP5
20	BMP6	Asn
					85					

	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
25	mOP-1
	hOP-2	...	His	Lys
	mOP-2	...	His	Lys
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val
	Vgl	His	...	Glu	Ala	...	Asp
30	Vgr-1
	CBMP-2A	Asn	...	Gln	Asp	Glu
	CBMP-2B	Asn	...	Gln	Glu	Glu
	BMP3	Val	...	Pro	Thr	...	Glu

	GDF-1	Gln	...	Glu	Asp	Asp
	60A	Ile	...	Lys
	BMP5
	BMP6	Trp
5		90					95		
	hOP-1	Ala	Cys	Gly	Cys	His			
	mOP-1			
10	hOP-2			
	mOP-2			
	DPP	Gly	Arg			
	Vgl	Glu	Arg			
	Vgr-1			
15	CBMP-2A	Gly	Arg			
	CBMP-2B	Gly	Arg			
	BMP3	Ser	...	Ala	...	Arg			
	GDF-1	Glu	Arg			
	60A	Ser			
20	BMP5	Ser			
	BMP6			
		100							

**Between residues 56 and 57 of BMP3 is a Val residue;
 between residues 43 and 44 of GDF-1 lies
 25 the amino acid sequence Gly-Gly-Pro-Pro.

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP-1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or 30 "similarity") with the hOP-1 sequence, where "homology" 35

or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed.

5 Res. Fd'n, Washington D.C. 1979.)

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater 10 than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP-1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the 15 Drosophila 60A protein. Accordingly, in still another preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and 20 accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding 25 position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

30 II. Formulations and Methods for Administering Therapeutic Agents

The morphogens may be provided to an individual by any suitable means, preferably directly, parenterally 35 or orally. Where the morphogen is to be provided

directly (e.g., locally, as by injection, to a bone tissue site), or parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, 5 intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to 10 delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The 15 aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, 20 to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. 25 For example, association of the mature dimer with the pro domain of the morphogen increases solubility of the protein significantly. In fact, the endogenous protein is thought to be transported in this form. Another molecule capable of enhancing solubility and 30 particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for oral or parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences,

- 5 (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include
10 glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, lactide and lactide/glycolide copolymers, may be useful excipients
15 to control the release of the morphogen in vivo. Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for
20 inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a
25 gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

30

Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins readily are degraded by digestive enzymes
35 and acids in the mammalian digestive system before they

can be absorbed into the bloodstream. However, the morphogens described herein typically are acid-stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590.) In addition, at least one morphogen, OP-1, 5 has been identified in bovine mammary gland extract, colostrum and milk (see Example 10, below) as well as saliva. Moreover, the OP-1 purified from mammary gland extract has been shown to be morphogenically active. Specifically, this protein has been shown to induce 10 endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in U.S. Pat. No. 4,968,590. In addition, endogenous morphogen also has been detected in the 15 bloodstream (see Example 11). These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly 20 soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with the pro domain of the intact sequence and/or by association with one or more milk components. 25 Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo, including, for example, part or all of a morphogen pro domain, and casein, as described above.

30

The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to bone tissue. For example, tetracycline and diphosphonates 35 are known to bind to bone mineral, particularly at

zones of bone remodeling, when they are provided systemically in a mammal. Alternatively, an antibody or other binding protein that interacts specifically with a surface molecule on bone tissue cells also may 5 be used. Such targeting molecules further may be covalently associated to the morphogen or morphogen-stimulating agent with, for example, an acid labile bond such as an Asp-Pro linkage, using standard chemical means well known in the art. Because the 10 local environment at bone remodeling sites is acidic, acid-labile linkages are expected to be preferentially cleaved at these sites, yielding active morphogen or morphogen-stimulating agent at the desired site. Useful targeting molecules may be designed, for 15 example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal 20 active domains. By contrast, the sequences diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain may be morphogen-specific. As described above, it is also known that the various morphogens identified to date 25 are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the body, selected morphogens typically act on a given tissue. Accordingly, part or all of pro domains, which 30 have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct 35 the morphogen associated with the pro domain to that

tissue. Accordingly, another useful targeting molecule for targeting morphogen to bone tissue is part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1, BMP2 or BMP4, all of which 5 proteins are found naturally associated with bone tissue.

Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in 10 combination with other molecules known to have a beneficial effect on maintaining appropriate bone remodeling cycles in an individual at risk for excessive bone loss. Examples of useful cofactors include vitamin D₃, calcitonin, prostaglandins, 15 parathyroid hormone, dexamethasone, estrogen and IGF.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and 20 carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of 25 powders, nasal drops, or aerosols.

The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which 30 provide appropriate concentrations of a morphogen to bone tissue for a time sufficient to inhibit loss of bone mass and/or to stimulate bone formation in individuals suffering from metabolic bone diseases and other bone remodeling disorders as described above.

Therapeutic concentrations also are sufficient to repair fractures and other defects in skeletal microstructure, and to enhance maintenance of appropriate bone mass in developing juveniles and 5 adults, including protecting individuals at risk for bone mass deterioration.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a 10 therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug 15 to be administered also is likely to depend on such variables as the type and extent of bone loss or defect, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound 20 excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 25 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μ g/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given in all cases is between 2-20 μ g of protein per kilogram weight of the patient per day. 30 Currently preferred dose ranges for local injection of soluble morphogen to bone tissue are 0.1-50 μ g morphogen/injection. No obvious morphogen-induced

pathological lesions are induced when mature morphogen (e.g., OP-1, 20 µg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 µg systemic injections of morphogen (e.g., OP-1) injected 5 daily for 10 days into normal newborn mice does not produce any gross abnormalities.

III. Examples

10 Example 1. Identification of Morphogen-Expressing Tissue

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed 15 in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA 20 transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or 25 immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

30 Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high 35 sequence homology in their active, C-terminal domains,

the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein.

- 5 Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly
- 10 useful Vgr-1-specific probe sequence is the Pvull-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence).
- 15 Similarly, particularly useful mOP-1-specific probe sequences are the BstX1-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain;
- 20 and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16)
- 25 or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

- Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in
- 30 mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology
 - 35 such as by the method of Chomczyaski et al. ((1987)

Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 µg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm²). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardt's, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in co-pending USSN 752,764, and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123, and Ozkaynak, et al. (1992) (JBC, in press), the disclosures of which are incorporated herein by reference. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart

tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed primarily in brain tissue. To 5 date, OP-2 appears to be expressed primarily in early embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 17-day embryos and is not 10 detected in post-natal animals.

Example 2. Mitogenic Effect of Morphogen on Rat and Human Osteoblasts

15 The ability of a morphogen to induce proliferation of osteoblasts may be determined in vitro using the following assay. In this and all examples involving osteoblast cultures, rat osteoblast-enriched primary cultures preferably are used. Although these cultures 20 are heterogeneous in that the individual cells are at different stages of differentiation, the culture is believed to more accurately reflect the metabolism and function of osteoblasts in vivo than osteoblast culture obtained from established cell lines. Unless otherwise 25 indicated, all chemicals referenced are standard, commercially available reagents, readily available from a number of sources, including Sigma Chemical, Co., St. Louis; Calbiochem, Corp., San Diego, and Aldrich Chemical Co., Milwaukee.

30 Rat osteoblast-enriched primary cultures were prepared by sequential collagenase digestion of newborn suture-free rat calvaria (e.g., from 1-2 day-old animals, Long-Evans strain, Charles River Laboratories, 35 Wilmington, MA), following standard procedures, such as

are described, for example, in Wong et al., (1975) PNAS 72:3167-3171. Rat osteoblast single cell suspensions then were plated onto a multi-well plate (e.g., a 48 well plate) at a concentration of 50,000 osteoblasts 5 per well in alpha MEM (modified Eagle's medium, Gibco, Inc., Long Island) containing 10% FBS (fetal bovine serum), L-glutamine and penicillin/streptomycin. The cells were incubated for 24 hours at 37°C, at which time the growth medium was replaced with alpha MEM 10 containing 1% FBS and the cells incubated for an additional 24 hours so that cells were in serum-deprived growth medium at the time of the experiment.

The cell culture then was divided into three 15 groups: (1) wells which received 0.1, 1.0, 10.0, 40 and 80.0 ng of morphogen; (2) wells which received 0.1, 1.0, 10.0 and 40 ng of a local-acting growth factor; and (3) the control group, which received no growth factors. In this example, OP-1 was the morphogen 20 tested, and TGF- β was the local-acting growth factor. The cells then were incubated for an additional 18 hours after which the wells were pulsed with 2 μ Ci/well of 3 H-thymidine and incubated for six more hours. The excess label then was washed off with a 25 cold solution of 0.15 M NaCl, 250 μ l of 10% tricholoracetic acid then was added to each well and the wells incubated at room temperature for 30 minutes. The cells then were washed three times with cold distilled water, and lysed by the addition of 250 μ l of 30 1% sodium dodecyl sulfate (SDS) for a period of 30 minutes at 37°C. The cell lysates then were harvested using standard means well known in the art, and the incorporation of 3 H-thymidine into cellular DNA was determined by liquid scintillation as an indication 35 of mitogenic activity of the cells. The results, shown

in FIG. 1, demonstrate that OP-1 (identified in the figure by squares) stimulates ^3H -thymidine incorporation into DNA, and thus promotes osteoblast cell proliferation. The mitogenesis stimulated by 5 40 ng of OP-1 in serum-free medium was equivalent to the mitogenic effect of 10% fresh serum alone. By contrast, the effect of TGF- β (indicated by diamonds in Fig. 1) is transient and biphasic. At high concentrations, TGF- β has no significant effect on 10 osteoblast cell proliferation. This system may be used to test other morphogens for their effect on cell proliferation.

The in vitro effect of a morphogen on osteoblast 15 proliferation also was tested on human primary osteoblasts (obtained from bone tissue of a normal adult patient and prepared as described above) and on osteosarcoma-derived cells, and in all cases induced cell proliferation. In addition, similar experiments, 20 performed using BMP4 (BMP2B) and BMP3 shows these morphogens also can stimulate osteoblast proliferation and growth. (See Chen et al., (1991) J. Bone and Min. Res. 6: 1387-1393, and Vukicevic, (1989) PNAS 86: 8793-8797.)

25

The effect of a given morphogen on bone cell growth and/or development also may be tested using a variety of bone cell markers: e.g., collagen synthesis, alkaline phosphatase activity, parathyroid 30 hormone-mediated cyclic AMP (cAMP) production, osteocalcin synthesis, and by assessing the rate of mineralization in osteoblasts. Of these, alkaline phosphatase activity, parathyroid hormone-mediated cAMP production, osteocalcin synthesis and mineralization 35 promotion are specific markers for the differentiated

osteoblast phenotype. Experimental systems for testing these parameters as well as collagen synthesis are described below in Examples 3-7. In all cases morphogen alone stimulated expression of these 5 phenotype-specific markers. In Examples 3-7 OP-1 was the morphogen tested. Similar experiments, performed using BMP4 (BMP2B) shows that this morphogen also induces osteoblast differentiation. (See Chen, et al. (1991) T. Bone and Min. Res. 6: 1387-1392, and 10 Vukicevic, (1989) PNAS 86: 8793-8797.)

Example 3. Effect of Morphogen on Collagen Synthesis
in Rat Osteoblasts

15

The effect of a morphogen on collagen production in rat osteoblasts in vitro may be determined as follows.

Rat osteoblasts were prepared and cultured in a 20 multi-well plate as described for Example 2. In this example a 24-well plate was used. The cultured cells then were divided into three groups: (1) wells which received 1, 10 or 40 ng of morphogen per ml of medium; (2) wells which received 1, 10 or 40 ng of a 25 local-acting growth factor per ml of medium; and (3) a control group which received no growth factors. In this example, OP-1 was the morphogen tested, and TGF- β was the local-acting growth factor.

30 The samples were incubated for 68 hours at 37°C with 5% CO₂ in a humidified incubator. Twenty-five (25) μ Ci of ³H proline were added into each well and incubated for six additional hours. The cells then were frozen at -20°C until the collagen assay was performed. The 35 cells then were assayed for collagen production by

detecting incorporation of ^3H -proline into total collagenase-digestible protein (CDP). The results, shown in FIG. 2, demonstrate that OP-1 stimulates type-I collagen synthesis, as measured by ^3H -proline incorporation into total CDP. Thus, OP-1 promotes collagen synthesis in vitro by preosteoblasts and mature osteoblasts.

10 Example 4. Alkaline Phosphatase Induction of Osteoblasts by Morphogen

4.1 Morphogen-specific Alkaline Phosphatase Induction

15

Since alkaline phosphatase production is an indicator of bone formation by differentiated, functional osteoblasts, a morphogen may be assessed for its potential osteogenic effects using this osteoblast marker in the following in vitro test system.

Rat osteoblasts were prepared and cultured in a multi-well plate as described for Example 2. In this example a 24-well plate was used. The cultured cells 25 then were divided into three groups: (1) wells which received varying concentrations of morphogen; (2) wells which received varying concentrations of a local-acting growth factor; and (3) a control group which received no growth factors. In this example OP-1 was the 30 morphogen tested at the following concentrations: 0.1, 1.0, 10.0, 40.0 or 80.0 ng/ml medium; and TGF- β was the local-acting growth factor, tested at 0.1, 1.0, 10.0, 40.0 or 80.0 ng/ml medium. The cells then were 35 incubated for 72 hours. After the incubation period the cell layer was extracted with 0.5 ml of 1% Triton

X-100. The resultant cell extract was centrifuged, 100 μ l of the extract was added to 90 μ l of paranitrosophenylphosphate (PNPP)/glycerine mixture and incubated for 30 minutes in a 37°C water bath and the reaction stopped with 100 μ l NaOH. The samples then were run through a plate reader (e.g., Dynatech MR700 plate reader, and absorbance measured at 400 nm, using p-nitrophenol as a standard) to determine the presence and amount of alkaline phosphate activity. Protein concentrations were determined by the Biorad method. Alkaline phosphatase activity was calculated in units/ μ g protein, where 1 unit=1 nmol p-nitrophenol liberated/30 minutes at 37°C.

15 The results, shown in FIG. 3, illustrate that morphogen alone stimulates the production of alkaline phosphatase in osteoblasts, and thus promotes the growth and expression of the osteoblast differentiated phenotype. In the figure, squares represent OP-1 concentrations, and diamonds represent TGF- β concentrations.

25 4.2. Long Term Effect of Morphogen on the Production of Alkaline Phosphatase by Rat Osteoblasts

30 In order to determine the long term effect of a morphogen on the production of alkaline phosphatase by rat osteoblasts, the following assay may be performed.

35 Rat osteoblasts were prepared and cultured in multi-well plates as described in Example 2. In this example six sets of 24 well plates are plated with 50,000 rat osteoblasts per well. The wells in each

plate, prepared as described above, then were divided into three groups: (1) those which received 1 ng of morphogen per ml of medium; (2) those which received 40 ng of morphogen/ml of medium; and (3) those which 5 received 80 ng of morphogen/ml of medium. Each plate then was incubated for different lengths of time: 0 hours (control time), 24 hours, 48 hours, 96 hours, 10 120 hours and 144 hours. After each incubation period, the cell layer was extracted with 0.5 ml of 1% Triton X-100. The resultant cell extract was centrifuged, and alkaline phosphatase activity determined as for Example 4, using paranitrosophenylphosphate (PNPP). The results, shown in FIG. 4, illustrate that morphogen alone stimulates the production of alkaline phosphatase 15 in osteoblasts, that increasing doses of OP-1 further increase the level of alkaline phosphatase production, and that the morphogen-stimulated elevated levels of alkaline phosphatase in the treated osteoblasts lasts for an extended period of time. In the figure, circles 20 represent 1 ng OP-1; squares, 40 ng OP-1; and diamonds, 80 ng OP-1.

Example 5. Morphogen-Induced Parathyroid Hormone
Mediated cAMP Production in Rat Osteoblasts

The effect of a morphogen on parathyroid hormone-mediated cAMP production in rat osteoblasts in vitro may be determined as follows.

30 Rat osteoblasts were prepared and cultured in a multiwell plate as described for Example 2 above. In this example a 24-well plate was used. The cultured cells then were divided into three groups: (1) wells 35 which received varying concentrations of morphogen (in

this example, OP-1, at 1.0, 10.0 and 40.0 ng/ml medium); (2) wells which received varying concentrations of a local-acting growth factor (in this example, TGF- β , at 0.1, 1.0, and 5.0 ng/ml medium); and 5 (3) a control group which received no growth factors. The plate was then incubated for another 72 hours. At the end of the 72 hours the cells were treated with medium containing 0.5% bovine serum albumin (BSA) and 1mM 3-isobutyl-1-methyl xanthine for 20 minutes 10 followed by the addition into half of the wells of human recombinant parathyroid hormone (hPTH, Sigma, St. Louis) at a concentration of 200ng/ml for 10 minutes. The cell layer was extracted from each well with 0.5 ml of 1% Triton X-100. The cAMP levels 15 were then determined using a radioimmunoassay kit (Amersham, Arlington Heights, Illinois). The results, shown in FIG. 5, demonstrate that morphogen alone stimulates an increase in the PTH-mediated cAMP response, and thus promotes the growth and expression 20 of the osteoblast differentiated phenotype.

Example 6. Effect of Morphogen on Osteocalcin Synthesis and the Rate of Mineralization by Osteoblasts in Culture

Osteocalcin is a bone-specific protein synthesized by osteoblasts which plays an integral role in the rate of bone mineralization in vivo. Circulating levels of 30 osteocalcin in serum are used as a marker for osteoblast activity and bone formation in vivo. Induction of osteocalcin synthesis in osteoblast-enriched cultures can be used to assay morphogen efficacy in vitro.

Rat osteoblasts are prepared and cultured in a multi-well plate as for Example 2. In this example cells were cultured in a 24-well plate. In this experiment the medium was supplemented with 10%FBS, and

5 on day 2, cells were fed with fresh medium supplemented with fresh 10 mM β -glycerophosphate (Sigma, Inc.). Beginning on day 5 and twice weekly thereafter, cells were fed with a complete mineralization medium containing all of the above components plus fresh L(+)-

10 ascorbate, at a final concentration of 50 μ g/ml medium. Morphogen then was added to the wells directly. In this example, OP-1 in 50% acetonitrile (or 50% ethanol) containing 0.1% trifluoroacetic acid (TFA) was added at no more than 5 μ l morphogen/ml medium. Control wells

15 received solvent vehicle only. The cells then were re-fed and the conditioned medium sample diluted 1:1 in standard radioimmunoassay buffer containing standard protease inhibitors and stored at -20° C until assayed for osteocalcin. Osteocalcin synthesis then was

20 measured by standard radioimmunoassay using a commercially available rat osteocalcin-specific antibody.

Mineralization was determined on long term cultures (13 day) using a modified von Kossa staining technique on fixed cell layers: cells were fixed in fresh 4% paraformaldehyde at 23° C for 10 mn, following rinsing cold 0.9% NaCl. Fixed cells then were stained for endogenous alkaline phosphatase at pH 9.5 for 10 min,

25 30 using a commercially available kit (Sigma, Inc.) Purple stained cells then were dehydrated with methanol and air dried. after 30 min incubation in 3% AgNO₃, in

the dark, H₂O-rinsed samples were exposed for 30 sec to 254 nm UV light to develop the black silver-stained phosphate nodules. Individual mineralized foci (at least 20 μ m in size) were counted under a dissecting 5 microscope and expressed as nodules/culture (see Fig. 6B).

As can be seen in Fig. 6A OP-1 stimulates osteocalcin synthesis in osteoblast cultures. The 10 increased osteocalcin synthesis in response to OP-1 is dose dependent and showed a 5-fold increase over the basal level using 25 ng of OP-1/10 ml medium after 13 days of incubation. The enhanced osteocalcin synthesis also can be confirmed by detecting the elevated 15 osteocalcin mRNA message (20-fold increase) using a rat osteocalcin-specific probe. In addition, the increase in osteocalcin synthesis correlates with increased mineralization in long term osteoblast cultures as determined by the appearance of mineral nodules 20 (compare Fig. 6A and 6B.) OP-1 increases the initial mineralization rate about 20-fold compared to untreated cultures. Similar experiments performed using TGF- β indicate that TGF- β does not induce osteocalcin synthesis or promote the mineralization process. Thus, 25 morphogen alone promotes the growth and expression of the osteoblast differentiated phenotype.

Example 7. Effect of Morphogen on Bone Derived Growth Factor Induction in vitro
30

IGF-I and IGF-II are bone-derived growth factors involved in coupling bone formation with bone resorption in the bone remodeling cycle. The effect of

morphogen on the production of these and other bone-derived growth factors, including TGF- β , may be evaluated using the following procedure.

5 Rat or human osteoblasts were prepared and cultured in a multiwell plate as for Example 2. The wells of the plate were divided in to groups in which different concentrations of morphogen were added (e.g., 0, 1, 10, and 100 ng). In this example, OP-1 was the morphogen
10 used. The plate then was incubated for a prescribed period of time, e.g., 72 hours, and the level of IGF detected, e.g., by immunolocalization, using a commercially available antibody specific for IGFs. OP-1 induced the level of both IGF-I and IGF-II significantly. Greater than six fold IGF-I and two fold IGF-II were induced following exposure to 100 ng OP-1/ml. In addition, OP-1 stimulated production of the IGF-I stimulating factor, BP3 (IGF-I binding protein 3).

20

Example 8. Effect of Morphogen on Trabecular Bone in Ovariectomized (OVX) Rats

As indicated above, serum alkaline phosphatase and
25 osteocalcin levels are indicators of bone formation within an individual. In order to determine the effect of a morphogen on bone production *in vivo*, these parameters are measured under conditions which promote osteoporosis, e.g., wherein osteoporosis is induced by
30 ovary removal in rats.

Forty Long-Evans rats (Charles River Laboratories, Wilmington) weighing about 200g each are ovariectomized (OVX) using standard surgical procedures, and ten rats
35 are sham-operated. The ovariectomy of the rats

produces an osteoporotic condition within the rats as a result of decreased estrogen production. Food and water are provided ad libitum. Eight days after ovariectomy, the rats, prepared as described above, 5 were divided into five groups: (A), 10 sham-operated rats; (B), 10 ovariectomized rats receiving 1 ml of phosphate-buffered saline (PBS) i.v. in the tail vein; (C) 10 ovariectomized rats receiving about 1 mg of 10 $17\beta E_2$ (17β -estradiol E_2) by intravenous injection through the tail vein; (D) 9 ovariectomized rats receiving daily injections of approximately $2\mu g$ of morphogen by tail vein for 22 days; and (E) 9 15 ovariectomized rats receiving daily injections of approximately $20\mu g$ of morphogen by tail vein for 22 days. In this example, OP-1 was the morphogen tested.

On the 15th and 21st day of the study, each rat was injected with 5 mg of tetracycline, and on day 22, the 20 rats were sacrificed. The body weights, uterine weights, serum alkaline phosphate levels, serum calcium levels and serum osteocalcin levels then were determined for each rat. The results are shown in Tables III and IV.

Body Weights, Uterine Weights and Alkaline Phosphatase

Group	<u>Body Weights</u> (g)	<u>Uterine Weights</u> (g)	<u>Alk. Phosphatase</u> (U/L)
5 A-SHAM	250.90 \pm 17.04	0.4192 \pm 0.10	43.25 \pm 6.11
B-OVX+PBS	273.40 \pm 16.81	0.1650 \pm 0.04	56.22 \pm 6.21
C-OVX+E2	241.66 \pm 21.54	0.3081 \pm 0.03	62.66 \pm 4.11
D-OVX+OP-1 (2 μ g)	266.67 \pm 10.43	0.1416 \pm 0.03	58.09 \pm 12.97
10 E-OVX+OP-1 (20 μ g)	272.40 \pm 20.48	0.1481 \pm 0.05	66.24 \pm 15.74

TABLE IV15 Serum Calcium and Serum Osteocalcin Levels

Group	<u>Serum Calcium</u> (ng/dl)	<u>Serum Osteocalcin</u> (ng/ml)
20 A-SHAM	8.82 \pm 1.65	64.66 \pm 14.77
B-OVX+PBS	8.95 \pm 1.25	69.01 \pm 10.20
C-OVX+E2	9.20 \pm 1.39	67.13 \pm 17.33
D-OVX+OP-1 (2 μ g)	8.77 \pm 0.95	148.50 \pm 84.11
25 E-OVX+OP-1 (20 μ g)	8.67 \pm 1.94	182.42 \pm 52.11

The results presented in Table III and IV show that intravenous injection of morphogen into ovariectomized rats produces a significant increase in serum alkaline phosphatase and serum osteocalcin levels and demonstrates that systemic administration of the morphogen stimulates bone formation in osteoporotic bone.

Example 9. Histomorphometric Analysis of Morphogen on the Tibia Diaphysis in Ovariectomized (OVX) Rats

- 5 Fifteen female Long-Evans rats weighing about 160 g were ovariectomized (OVX) to produce an osteoporotic condition and five rats were sham operated (Charles River Laboratories, Wilmington, MA.) as described for Example 8. Food and water were provided ad libitum.
- 10 Twenty-two days after ovariectomy, the rats were divided into four groups: (A) sham-operated (1 ml of PBS by intravenous injection through tail vein (5 rats); (B) OVX, into which nothing was injected (5 rats); (C) OVX, receiving about 1 mg of $17\beta E_2$ by
- 15 intravenous injection through the tail vein (5 rats), and (D) OVX, receiving about 1 μ g of morphogen by intravenous injection through the tail vein (5 rats). In this example, OP-1 was morphogen tested.
- 20
- The rats were injected daily as described for seven days, except no injections were given on the thirteenth day. The rats then were sacrificed on the nineteenth day. The tibial diaphyseal long bones then were
- 25 removed and fixed in ethanol and histomorphometric analysis was carried out using standard procedures well known in the art. The results are shown in Table V.

Table V

MEASUREMENT	(A) CONTROL	(B) OVX	(C) OVX + E ₂	(D) OVX + OP-1
5 Longitudinal Growth Rate ($\mu\text{m}/\text{day}$)	20.2 \pm 0.3	19.4 \pm 0.2	4.9 \pm 0.5	17.9 \pm 0.9
Cancellous Bone Volume (BV/TV, 10 bone vol/total vol)	20.2 \pm 1.5	13.0 \pm 1.6	13.7 \pm 2.1	16.6 \pm 1.8
Cancellous Bone Perimeter (mm)	16.2 \pm 1.8	9.6 \pm 0.9	11.5 \pm 1.1	12.2 \pm 0.7
15 Labeled Cancellous Perimeter (%)	35.5 \pm 1.5	51.9 \pm 5.6	58.0 \pm 4.2	39.2 \pm 1.9
Mineral Apposition Rate ($\mu\text{m}/\text{day}$)	1.76 \pm 0.14	2.25 \pm 0.16	1.87 \pm 0.08	1.86 \pm 0.20

20

The results presented in Table V confirm the results of Example 8, that intravenous injection of OP-1 into ovariectomized rats stimulates bone growth for bone which had been lost due to the drop in 25 estrogen within the individual rat. Specifically, the inhibition of cancellous bone volume in OVX rats is repaired by the systemically provided morphogen. In addition, in morphogen-treated rats the labelled cancellous perimeter and mineral apposition rate now 30 return to levels measured in the control, sham-operated rats. Moreover, morphogen treatment does not inhibit longitudinal bone growth, unlike estrogen treatment, which appears to inhibit bone growth significantly.

Accordingly, systemic administration of a morphogen in therapeutically effective concentrations effectively inhibits loss of bone mass in a mammal without inhibiting natural bone formation.

5

Example 10. Determination of the Presence of Morphogen in Body Fluids

OP-1 has been identified in saliva, human blood
10 serum, and various milk forms, including mammary gland extract, colostrum, and 57-day bovine milk. Moreoever, as described below, the body fluid extracted protein is morphogenically active. The discovery that the morphogen naturally is present in milk, together with
15 the known observation that mature, active OP-1 is acid-stable and protease-resistant, indicate that oral administration is a useful route for therapeutic administration of morphogen to a mammal. Oral administration typically is the preferred mode of
20 delivery for extended or prophylactic therapies. In addition, the identification of morphogen in all milk forms, including colostrum, indicates that the protein plays a significant role in tissue development, including skeletal development of juveniles (see
25 Example 13, below).

10.1 Morphogen Detection in Milk

OP-1 was partially purified from rat mammary gland
30 extract and bovine colostrum and 57 day milk by passing these fluids over a series of chromatography columns: (e.g., cation-exchange, affinity and reverse phase). At each step the eluant was collected in fractions and these were tested for the presence of OP-1 by standard
35 immunoblot. Immunoreactive fractions then were

combined and purified further. The final, partially purified product then was examined for the presence of OP-1 by Western blot analysis using OP-1-specific antisera, and tested for in vivo and in vitro activity.

5

OP-1 purified from the different milk sources were characterized by Western blotting using antibodies raised against OP-1 and BMP2. Antibodies were prepared using standard immunology protocols well known in the art, and as described generally in Example 14, below, using full-length E. coli-produced OP-1 and BMP2 as the immunogens.

As shown in Fig. 7 OP-1 purified from colostrum reacts with the anti-OP-1 antibody, but not with anti-BMP2 antibody. In Fig. 7 lane 1 contains reduced, purified, recombinantly-produced OP-1; lane 2 contains purified bovine colostrum, and lane 3 contains reduced COP-16, a biosynthetic construct having morphogenic activity and an amino acid sequence modeled on the proteins described herein, but having highest amino acid sequence homology with BMP2 (see US Pat. No. 5,011,691 for the COP-16 amino acid sequence.) In Fig. 7A the gel was probed with anti-OP-1 antibody; in Fig. 17B, the gel was probed with anti-BMP2 antibody. As can be seen in the figure, anti-OP-1 antibody hybridizes only with protein in lanes 1 and 2, but not 3; while anti-BMP2 antibody hybridizes with lane 3 only.

30

Column-purified mammary gland extract and 57-day milk also reacts specifically with anti-OP-1 antibodies, including antibody raised against the full length E. coli OP-1, full length mammalian-produced 5 OP-1, and the OP-1 Ser-17-Cys peptide (e.g., the OP-1 N-terminal 17 amino acids).

The morphogenic activity of OP-1 purified from mammary gland extract was evaluated in vivo as follows.

10 A sample was prepared from each OP-1 immunoreactive fraction of the mammary gland extract-derived OP-1 final product by lyophilizing a portion (33%) of the fraction and resuspending the protein in 220 μ l of 50% acetonitrile/0.1% TFA. After vortexing, 25 mg of 15 collagen matrix was added. The samples were lyophilized overnight, and implanted in Long Evans rats (Charles River Laboratories, Wilmington, MA, 28-35 days old). Each fraction was implanted in duplicate. For details of the collagen matrix implantation procedure, 20 see, for example, U.S. Pat. No. 4,968,590, hereby incorporated by reference. After 12 days, the implants were removed and evaluated for new bone formation by histological observation.

25 The results are presented in Fig. 8A, where "% activity" refers to the percent of bone formation/total area covered by bone in the histology sample. In the figure, solid bars represent implants using mammary extract-derived OP-1, each bar corresponding to an 30 immunoreactive fraction of the purified product, the fraction number being indicated on the x-axis. The hatched bar represents an implant using recombinantly produced OP-1 (600 ng). As can be seen in the figure, all immunoreactive fractions are osteogenically active.

Similarly, the morphogenic activity of OP-1 purified from mammary gland extract was evaluated in vitro by measuring alkaline phosphatase activity in vitro using the following assay. Test samples were 5 prepared as for the in vivo assay, using 15-20% of individual immunoreactive fractions collected from the final product. Alkaline phosphatase activity was tested as described above in Example 4. The results, presented in Fig. 8B, indicate that the immunoreactive 10 fractions can stimulate alkaline phosphatase activity in vitro. Moreover, the activity correlates well with that produced by highly purified, recombinantly produced, OP-1. In Fig. 8B solid bars represent assays performed with mammary gland-purified OP-1, each bar 15 corresponding to an immunoreactive fraction of column-purified OP-1, the fraction numbers being indicated on the x-axis; the hatched bar represents the assay performed with purified, recombinantly-produced OP-1 (100 ng/ml); and the cross-hatched bar represents 20 background.

10.2 Morphogen Detection in Serum

Morphogen may be detected in serum using morphogen-specific antibodies. The assay may be performed using 25 any standard immunoassay, such as Western blot (immunoblot) and the like. Preferably, the assay is performed using an affinity column to which the morphogen-specific antibody is bound and through which 30 the sample serum then is poured, to selectively extract the morphogen of interest. The morphogen then is eluted. A suitable elution buffer may be determined empirically by determining appropriate binding and elution conditions first with a control (e.g., 35 purified, recombinantly-produced morphogen.) Fractions

- then are tested for the presence of the morphogen by standard immunoblot, and the results confirmed by N-terminal sequencing. Preferably, the affinity column is prepared using monoclonal antibodies. Morphogen 5 concentrations in serum or other fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation of conjugated antibody.
- 10 Presented below is a sample protocol for identifying OP-1 in serum. Following this general methodology other morphogens may be detected in body fluids, including serum. The identification of morphogen in serum further indicates that systemic 15 administration is a suitable means for providing therapeutic concentrations of a morphogen to an individual, and that morphogens likely behave systemically as endocrine-like factors. Finally, using this protocol, fluctuations in endogenous morphogen 20 levels can be detected, and these altered levels may be used as an indicator of bone tissue dysfunction. Alternatively, fluctuations in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard northern blot analysis as described 25 in Example 1, or by in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA, and standard RNA hybridization protocols well described in the art and described generally in Example 1.
- 30 OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and 35 described generally in Example 14, was immobilized by

passing the antibody over an agarose-activated gel (e.g., Affi-Gel™, from Bio-Rad Laboratories, Richmond, CA, prepared following manufacturer's instructions) and used to purify OP-1 from serum. Human serum then was
5 passed over the column and eluted with 3M K-thiocyanate. K-thiocyanante fractions then were dialyzed in 6M urea, 20mM PO₄, pH 7.0, applied to a C8 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly
10 produced OP-1 homodimers elute between 20-22 minutes. Fractions then were collected and tested for the presence of OP-1 by standard immunoblot using an OP-1 specific antibody as for Example 10.A. Fig. 9 is an immunoblot showing OP-1 in human sera under reducing
15 and oxidized conditions. In the figure, lanes 1 and 4 are OP-1 standards, run under oxidized (lane 1) and reduced (lane 4) conditions. Lane 5 shows molecular weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are human sera OP-1, run under oxidized (lane 2) and
20 reduced (lane 3) conditions.

Morphogens may be used in diagnostic applications by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value,
25 with fluctuations in fluid morphogen levels indicating a change in the status of bone tissue. Alternatively, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein
30 capable of interacting specifically with the endogenous morphogen antibody. Detected fluctuations in the levels of the endogenous antibody may be used as indicators of a change in tissue status.

Example 11. Morphogen-induced Periosteal and Endosteal Bone Formation

Osteoclast-induced bone resorption occurs primarily at the endosteal surface of bone tissue. Accordingly, in bone remodeling disorders the marrow cavity is enlarged unnaturally, weakening the weight bearing capacity of the remaining bone. The following example provides means for evaluating the ability of the morphogens described herein to increase endosteal and periosteal bone mass in a mammal. In this example, both periosteal and endosteal bone formation are induced by direct injection of a morphogen in a biocompatible solution directly to the bone tissue. As demonstrated below, morphogens can induce new bone formation and increase bone mass at both surfaces when provided to the bone by direct injection. Direct injection may be a preferred mode of administration for providing therapeutically effective concentrations to reduce an enlarged marrow cavity, and/or to repair fractures and other damage to bone tissue microstructure.

Morphogen was provided to either the periosteum (outer or peripheral bone surface) and endosteum (interior bone surface, e.g., that surface lining the marrow cavity) of a rat femur by a single injection in each case. Specifically, morphogen (e.g., OP-1, 2-20 µg) was provided to the bone tissue as an insoluble colloidal suspension in phosphate-buffered saline. Endosteal injection was performed through a microhole made with a hand-held orthopedic drill. After 7 days, the treated bones were removed and prepared for histological evaluation as described in U.S. Pat. No. 4,968,590. As little as 2 µg morphogen is

sufficient to induce new bone formation at the site of injection within 4-7 days. In addition, bone induction is dose-dependent. Photomicrographs of the histology are presented in Fig. 10. In the figure, "ob" means
5 old bone, "bm" means bone marrow, "nb" means new bone, and "m" means muscle. Fig.10A shows new bone formed following injection of morphogen to the endosteal surface. As can be seen in the figure, new bone has formed within the bone marrow cavity, filling in the
10 periphery of the cavity. Fig 10B shows new bone formed following injection of morphogen to the periosteal surface, replacing the muscle normally present.

15 Example 12. Effect of Morphogen on Bone Resorption

The effect of morphogen on bone resorption may be evaluated using rat osteoclasts on bovine bone slices, in the presence and absence of morphogen, and the
20 effect of morphogen on pit formation (resorption index) determined. Under standard conditions rat osteoclasts begin resorbing the bone tissue, causing pit formation on the bone slice surface. In this experiment OP-1 was the morphogen tested, at concentrations of 0, 5, 10,
25 20, 40, 50, and 100 ng/ml.

The results are presented in figure 11, where the resorption index is calculated as a percent of the control (e.g., bone resorption in the absence of
30 morphogen), calculated as the number of pits per a given slice surface area. Below 40 ng bone resorption is enhanced; above 40 ng, OP-1 has no apparent effect on bone resorption. The results highlight the integral role the morphogen plays in bone remodeling. OP-1 is

stored in bone tissue in vivo. In a normal bone remodeling cycle, the local concentration of OP-1 at the surface likely is low when osteoclasts begin resorbing bone, and the low concentration may enhance 5 and/or stimulate bone resorption. As resorption continues, the local concentration of OP-1 at the surface likely increases, to a concentration that no longer has an effect on osteoclasts, but continues to affect osteoblast growth and activity (see 10 Examples 2-7), stimulating bone growth.

In addition, morphogens can inhibit multinucleation of mononuclear phagocytic cells under conditions where these cells normally would be activated. For example, 15 in the absence of morphogen, an implanted substrate material (e.g., implanted subcutaneously) composed of, for example, mineralized bone, a ceramic such as titanium oxide or any other substrate that provokes multinucleated giant cell formation, rapidly becomes 20 surrounded by multinucleated giant cells, e.g., activated phagocytes stimulated to respond and destroy the foreign object. In the presence of morphogen however, the recruited cells remain in their mononuclear precursor form and the matrix material is 25 undisturbed. Figure 12 illustrates this effect of morphogens, in a schematic representation of histology results of a titanium oxide substrate implanted subcutaneously. In the figure, "mg" means multinucleated giant cells and "ob" means osteoblasts. The substrate 30 represented in Fig. 12B was implanted together with morphogen (OP-1) and newly formed osteoblasts are evident surrounding the substrate. By contrast, the substrate represented in Fig. 12A was implanted without morphogen and extensive multinucleated giant cell 35 formation is evident surrounding the substrate.

Accordingly, the morphogens' effect in inhibiting excessive bone mass loss in a mammal also may include inhibiting activation of these cells.

5 Example 13. Effect of Morphogen Neutralization on Bone Growth

The effect of the morphogens described herein on bone growth in developing mammals also may be evaluated
10 using neutralizing antibodies specific for particular morphogens and assessing the effect of these antibodies on bone development. Specifically, anti-morphogen monoclonal and/or polyclonal antibodies may be prepared using standard methodologies including, for example,
15 the protocol provided in Example 14, below.

Purified antibodies then are provided regularly to new born mice, e.g., 10-100 μ g/injection/day for 10-15 days. At 10 or 21 days, the mice are sacrificed and
20 the effect of morphogen on bone development assessed by body weight, gross visual examination and histology. In this example, anti-OP-1 antibodies were used. Morphogen neutralization significantly stunted body growth, including bone growth, as indicated by the
25 reduced body weight and reduced bone length of the treated mammals.

Similarly, morphogen activity may be assessed in fetal development in the mouse model using the
30 following assay. Single lip injections comprising 10-100 μ g/injection of morphogen-specific antibody are administered to pregnant female mice during each day of the gestation period and bone development in treated and control new mice evaluated by standard
35 histomorphometric analysis at birth. Similarly, single

lip injections also may be provided to juvenile and adult mice (e.g., 10-100 µg) over a prolonged time (e.g., 10-15 days) to evaluate the effect on bone growth and bone integrity and to evaluate the onset of 5 osteoporosis. The antibodies are anticipated to inhibit tissue morphogenesis, including bone growth and bone development, in the developing embryos.

10 Example 14. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level 15 of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the compound, in order to assess the effects of the compound on the cell. This can be accomplished by 20 detection of the morphogen either at the protein or RNA level. A detailed description also may be found in USSN 752,861, incorporated hereinabove by reference.

14.1 Growth of Cells in Culture
25

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult 30 rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 35 well or 24 well) according to conventional cell culture

- techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., 5 fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).
- 10 Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for morphogen production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor de novo morphogen synthesis, some cultures are labeled according to conventional procedures with an 15 ³⁵S-methionine/³⁵S-cysteine mixture for 6-24 hours and 20 then evaluated for morphogenic protein synthesis by conventional immunoprecipitation methods.

14.2 Determination of Level of Morphogenic Protein 25

- In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For 30 example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 μ g/100 μ l of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 35 96-well plate and incubated at 37°C for an hour. The

wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C.

5 The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μ l aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in

10 triplicate and incubated at 37°C for 30 min. After incubation, 100 μ l biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then

15 washed four times with BSB containing 0.1% Tween 20. 100 μ l strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates

20 are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50 μ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15 min. Then, 50 μ l amplifier (from the same

25 amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 μ l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1

30 standard curve is performed in parallel with the test samples.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100
35 ug/500 μ l E. coli-produced OP-1 monomer (amino acids

- 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 μ l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in
- 5 the same manner using incomplete Freund's Adjuvant.
- Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against OP-1 is detected in the serum using an ELISA assay.
- 10 Then, the rabbit is boosted monthly with 100 μ g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100 μ g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 μ g of OP-1 in incomplete adjuvant

20 and is given intraperitoneally. The mouse then receives a total of 230 μ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted

25 intraperitoneally with 100 μ g of OP-1 (307-431) and 30 μ g of the N-terminal peptide (Ser₂₉₃-Asn₃₀₉-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days

30 (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to commercially available myeloma cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim, Germany), and the cell fusion is plated and screened for OP-1-specific antibodies using

35 OP-1 (307-431) as antigen. The cell fusion and

monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

5

Other Embodiments

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

Other embodiments of the invention are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Thangavel Kuberasanpath
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(ii) TITLE OF INVENTION: TREATMENT TO
PREVENT LOSS OF AND/OR INCREASE
BONE MASS IN METABOLIC BONE DISEASE

(iii) NUMBER OF SEQUENCES: 33

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy Disk
(B) COMPUTER: IBM XT
(C) OPERATING SYSTEM: DOS 3.30
(D) SOFTWARE: PatentIn Release 1.0,
Version 1.25

(vi) CURRENT APPLICATION DATA:

(B) FILING DATE:

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- (B) FILING DATE: 30-AUG-1991

(viii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 667,274
- (B) FILING DATE: 11-MAR-1991

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 1
- (D) OTHER INFORMATION: Each Xaa
indicates one of the 20 naturally-
occurring L-isomer, α -amino acids
or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	1			5	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	10			15	
Xaa	Xaa	Xaa	Xaa	Xaa	Cys
	20			25	Xaa
Cys	Xaa	Xaa	Xaa	Xaa	Xaa
	30			35	Xaa
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	40			45	Xaa
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	55			60	Cys
Cys	Xaa	Xaa	Xaa	Xaa	Xaa
	65			70	Xaa

- 90 -

Xaa
75 80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
85 90
Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 2
- (D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Xaa Xaa Xaa
1 5
Xaa
10 15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
30 35

- 91 -

Xaa
40 45 50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70
Xaa
75 80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
85 90
Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 3
- (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Tyr Val Xaa Phe

1 5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
15 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Gly Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 4
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
1 5 10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
15
Xaa Ala Pro Xaa Gly Xaa Xaa Ala
20 25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
30 35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
40
Asn Xaa Xaa Asn His Ala Xaa Xaa
45 50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Gly Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: hOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
1				5				
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
				20		25		
Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
				30		35		
Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
				40			45	
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
				50				
Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
				55		60		
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
				65		70		
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
				75		80		
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
				85			90	

Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
						95		
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100						105		
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
						115		
110								
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
						125		
120								
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
						135		
130								
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: mOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln
1				5				
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala
						25		
20								
Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
					30		35	

Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
			40				45	
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
				50				
Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	55				60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
			65			70		
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
			75			80		
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
				85			90	
Val	His	Phe	Ile	Asn	Pro	Asp	Thr	Val
					95			
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	100				105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
			110			115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
			120			125		
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
				130			135	
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln
10					15			
Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
	20					25		
Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln
		30				35		
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
			40				45	
Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp
				50				
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
	55				60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
		65				70		
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75				80		
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
			85			90		
Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val
				95				
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
100					105			
Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120				125		
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130				135	
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: mOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln
1				5				
Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
10					15			
Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
20					25			
Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu
	30				35			
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val
		40				45		
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp
			50					
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
65					70			
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala
		75				80		
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu
				85			90	

Val His Leu Met Lys Pro Asp Val Val
95
Pro Lys Ala Cys Cys Ala Pro Thr Lys
100 105
Leu Ser Ala Thr Ser Val Leu Tyr Tyr
110 115
Asp Ser Ser Asn Asn Val Ile Leu Arg
120 125
Lys His Arg Asn Met Val Val Lys Ala
130 135
Cys Gly Cys His

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: CBMP2A(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro
15 20
Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu
25 30
Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser
35 40

- 100 -

Thr Asn His Ala Ile Val Gln Thr Leu Val Asn
45 50 55
Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys
60 65
Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
70 75
Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys
80 85
Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
90 95
Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
(A) NAME: CBMP2B(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser
1 5
Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
10 15
Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala
20 25
Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
30 35

Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
40 45
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
50 55 60
Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu
65 70
Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr
75 80
Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
85 90
Val Val Glu Gly Cys Gly Cys Arg
95 100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: DPP(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
15 20
Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
25 30

Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
35 40
Thr Asn His Ala Val Val Gln Thr Leu Val Asn
45 50 55
Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
60 65
Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
70 75
Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
80 85
Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
90 95
Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgl(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
1 5 10
Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
15 20
Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
25 30

Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
35 40
Ser Asn His Ala Ile Leu Gln Thr Leu Val His
45 50 55
Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
60 65
Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
70 75
Leu Phe Tyr Asp Asn Asn Asp Asn Val Val Leu
80 85
Arg His Tyr Glu Asn Met Ala Val Asp Glu Cys
90 95
Gly Cys Arg
100.

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Vgr-1(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
1 5 10
Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
15 20
Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
25 30

Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
35 40
Thr Asn His Ala Ile Val Gln Thr Leu Val His
45 50 55
Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
60 65
Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
70 75
Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
80 85
Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
90 95
Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids
- (B) TYPE: protein
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (F) TISSUE TYPE: BRAIN

(ix) FEATURE:

- (D) OTHER INFORMATION:
/product= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
1 5 10

Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
15 20 25

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
30 35 40

- 105 -

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
 45 50 55

Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
 60 65 70

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
 75 80 85

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
 90 95 100

Cys Arg
 105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa
 1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 49..1341
- (D) OTHER INFORMATION:/standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGCAGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCAGCG ATG CAC GTG
 Met His Val
 1

57

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	105
5 10 15	
CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	153
20 25 30 35	
GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	201
40 45 50	
CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	249
55 60 65	
CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG PrBo Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met	297
70 75 80	
CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Pro Gly	345
85 90 95	
GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly	393
100 105 110 115	
CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp	441
120 125 130	
ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe	489
135 140 145	
CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile	537
150 155 160	
CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp	585
165 170 175	
TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr	633
180 185 190 195	
CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu	681
200 205 210	

GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp 215 220 225	729
ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CCG CAC AAC CTG Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu 230 235 240	777
GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro 245 250 255	825
AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro 260 265 270 275	873
TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile 280 285 290	921
CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro 295 300 305	969
AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser 310 315 320	1017
AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys His Glu Leu Tyr Val Ser Phe 325 330 335	1065
CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355	1113
GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370	1161
AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385	1209
CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400	1257
ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415	1305

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430	1351
GAGAATTCAAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCCTC CCTATCCCCA ACTTTAAAGG TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTG ATCAGTTTT CAGTGGCAGC ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAAACAC GCATAAAAGAA AAATGCCGG GCCAGGTCAAT TGGCTGGAA GTCTCAGCCA TGCACGGACT CGTTTCCAGA GGTAATTATG AGCCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG GGCGTGGCAA GGGGTGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A	1411 1471 1531 1591 1651 1711 1771 1822

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 431 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (D) OTHER INFORMATION: /Product="OP1-PP"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala 1 5 10 15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser 20 25 30
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser 35 40 45
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu 50 55 60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro 65 70 75 80
Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly 85 90 95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
115 120 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145 150 155 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

V[Bal Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 1873 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

 - (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

 - (A) NAME/KEY: CDS
 - (B) LOCATION: 104..1393
 - (D) OTHER INFORMATION: /note= "MOP1 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCG CCCTCCGCTG CCACCTGGGG 600

CGGCGCGGGC CCGGTGCCCG GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115
Met His Val Arg
1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163
 Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
 5 10 15 20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211
 Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
 25 30 35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG
 Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
 40 45 50

- 111 -

GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro 55 60 65	307
CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu 70 75 80	355
GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln 85 90 95 100	403
GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro 105 110 115	451
TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val 120 125 130	499
ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro 135 140 145	547
CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu 150 155 160	595
GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile 165 170 175 180	643
CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val 185 190 195	691
CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser 200 205 210	739
CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr 215 220 225	787
GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu 230 235 240	835
CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu 245 250 255 260	883

GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met 265 270 275	931
GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser 280 285 290	979
ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn 295 300 305	1027
CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp 310 315 320	1075
CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp 325 330 335 340	1123
CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr 345 350 355	1171
TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala 360 365 370	1219
ACCB AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp 375 380 385	1267
ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser 390 395 400	1315
GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg 405 410 415 420	1363
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTTTCC TGAGACCCCTG Asn Met Val Val Arg Ala Cys Gly Cys His 425 430	1413
ACCTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
GGCACGTGAC GGACAAGATC CTACCAAGCTA CCACAGAAA CGCCTAAGAG CAGGAAAAAT	1653

GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCCTGGC GCTCTGAGTC TTTGAGGAGT	1713
AATCGCAAGC CTCGTTCAAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG	1773
TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833
GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATT	1873

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (D) OTHER INFORMATION: /product= "mOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 35 40 45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 85 90 95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110

Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 115 120 125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
 145 150 155 160

Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
165 170 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
180 185 190

Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
195 200 205

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
210 215 220

Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
225 230 235 240

Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
245 250 255

Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
260 265 270

Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
275 280 285

Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
290 295 300

Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
305 310 315 320

Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
340 345 350

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
355 360 365

Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
370 375 380

Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
385 390 395 400

Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
405 410 415

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1723 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Homo sapiens
 - (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 490..1696
- (D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCCCTGGA GCAACAGCTC	120
CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCATC GCCCCTGCCG TGCTCGGACC	180
GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240
CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
GACAGGTGTC GCGCGGCCGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
CGCCCCGCC CGCCGCCCGC CGCCCGCCGA GCCCAGCCTC CTTGCCGTG GGGCGTCCCC	420
AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GGCCCCCAGC TGAGCCGCC	480
CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	528
1 5 10	
CGC CTA TGC GCG CTG GGC GGG GGC CCC GGC CTG CGA CCC CCG CCC Ala Leu Cys Ala Leu Gly Gly Pro Gly Leu Arg Pro Pro Pro	576
15 20 25	
GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	624
30 35 40 45	
CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	672
50 55 60	

GC G C C G C C A C C C G C C T C C C G G C T G C C C G G C T C C G C C G C T C T C C T C A T G A l a P r o P r o A l a A l a S e r A r g L e u P r o A l a S e r A l a P r o L e u P h e M e t 65 70 75	720
C T G G A C C T G T A C C A C G C C A T G G C C G C G A C G A C G A C G A G G G C G C G L e u A s p L e u T y r H i s A l a M e t A l a G l y A s p A s p A s p G l u A s p G l y A l a 80 85 90	768
C C C G C G G A G C G G C C G C T G G G C C G C G C G C G C G C A C C T G G T C A T G A G C T T C G T T P r o A l a G l u A r g A r g L e u G l y A r g A l a A s p L e u V a l M e t S e r P h e V a l 95 100 105	816
A A C A T G G T G G G A G C G A G C C G T G C C G C A C C A G G A G C C C C A T T G G A s n M e t V a l G l u A r g A s p A r g A l a L e u G l y H i s G l n G l u P r o H i s T r p 110 115 120 125	864
A A G G A G G T T C C G C T T T G A C C T G A C C A G A T C C C G G C T G G G G A G G C G G G T C L y s G l u P h e A r g P h e A s p L e u T h r G l n I l e P r o A l a G l y G l u A l a V a l 130 135 140	912
A C A G C T G C G G A G T T C C G G A T T T A C A A G G T G G C C C A G C A T C C A C C T G C T C T h r A l a A l a G l u P h e A r g I l e T y r L y s V a l P r o S e r I l e H i s L e u L e u 145 150 155	960
A A C A G G A C C C T C C A C G T C A G C A T G T T C C A G G T G G G T C C A G G A G C A G T C C A s n A r g T h r L e u H i s V a l S e r M e t P h e G l n V a l V a l G l n G l u G l n S e r 160 165 170	1008
A A C A G G G A G T C T G A C T T G T T C T T G A T C T T C A G A C G C T C C G A G C T A s n A r g G l u S e r A s p L e u P h e P h e L e u A s p L e u G l n T h r L e u A r g A l a 175 180 185	1056
G G A G A C G A G G G C T G G T G G C T G G A T G T C A C A G A G T G A C T G C G l y A s p G l u G l y T r p L e u V a l L e u A s p V a l T h r A l a A l a S e r A s p C y s 190 195 200 205	1104
T G G T T G C T G A A G C G T C A C A A G G A C C T G G G A C T C C C G C C T C T A T G T G G A G T r p L e u L e u L y s A r g H i s L y s A s p L e u G l y L e u A r g L e u T y r V a l G l u 210 215 220	1152
A C T G A G G A C G G G C A C A G C G T G G A T C C T G G C C T G G C C G C T G C T G G G T T h r G l u A s p G l y H i s S e r V a l A s p P r o G l y L e u A l a G l y L e u L e u G l y 225 230 235	1200
C A A C G G G C C A C C G T C C C A A C A G C C T T G T G G T C A C T T T C A G G G l n A r g A l a P r o A r g S e r G l n G l n P r o P h e V a l V a l T h r P h e P h e A r g 240 245 250	1248
G C C A G T C C G A G T C C C A T C C G C A C C C C T C G G G C A G T G G A G G C C A C T G G A l a S e r P r o S e r P r o I l e A r g T h r P r o A r g A l a V a l A r g P r o L e u A r g 255 260 265	1296

AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu 270 275 280 285	1344
CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys 290 295 300	1392
CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315	1440
TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330	1488
TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345	1536
CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365	1584
TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys 385 390 395	1680
GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG Ala Cys Gly Cys His 400	1723

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 402 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
 (A) OTHER INFORMATION: /product= "hOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys 1 5 10 15
--

Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
20 25 30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
35 40 45

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
50 55 60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
85 90 95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
115 120 125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
130 135 140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
165 170 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
195 200 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
210 215 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
225 230 235 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
245 250 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
260 265 270

Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
275 280 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
290 295 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
325 330 335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
340 345 350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
370 375 380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
385 390 395 400

Cys His

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 93..1289
 - (D) OTHER INFORMATION: /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCAGGCACA GGTGCGCCGT CTGGTCCCTCC CCGTCTGGCG TCAGCCGAGC 50

CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT 104
Met Ala Met Arg
1

CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC 152
 Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly
 5 10 15 20

- 120 -

GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly 25 30 35	200
GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly 40 45 50	248
CTA CCG GGA CGG CCC CGA CCA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG Leu Pro Gly Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln 55 60 65	296
CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr 70 75 80	344
GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp 85 90 95 100	392
CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly 105 110 115	440
TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile 120 125 130	488
CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu 135 140 145	536
CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu 150 155 160	584
G TG GTC CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp 165 170 175 180	632
CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile 185 190 195	680
ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly 200 205 210	728
CTC CGC CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly 215 220 225	776

CTG GCT GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe 230 235 240	824
ATG GTA ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg 245 250 255 260	872
GCA GCG AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu 265 270 275	920
CCG CAC CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser 280 285 290	968
CGC GGC AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg 295 300 305	1016
GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala 310 315 320	1064
TAT TAC TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn 325 330 335 340	1112
GCC ACC AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro 345 350 355	1160
GAT GTT GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr 360 365 370	1208
TCT GTG CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His 375 380 385	1256
CGT AAC ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC Arg Asn Met Val Val Lys Ala Cys Gly Cys His 390 395	1309
TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAC CCTTCTATGT	1369
TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCCTGCTA	1429
AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGT TTCGGGGCTA TCACCCCCGCC	1489
CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA	1549

ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC	1609
CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGGC CGTGGAAATTC TAAACTAGAT	1669
GATCTGGGCT CTCTGCACCA TTCATTGTGG CAGTTGGAC ATTITTTAGGT ATAACAGACA	1729
CATACACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA	1789
AGAATCAGAG CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC	1849
AGGAGAACATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA	1909
AAAAAAAAAAC GGAATT	1926

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (D) OTHER INFORMATION: /product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys			
1	5	10	15
Ala Leu Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln			
20	25	30	
Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala			
35	40	45	
Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala			
50	55	60	65
Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala			
70	75	80	
Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg			
85	90	95	
Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr			
100	105	110	
Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr			
115	120	125	130

Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
135 140 145

Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
150 155 160

Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
165 170 175

Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
180 185 190

Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
195 200 205 210

Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
215 220 225

Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
230 235 240

Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
245 250 255

Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
260 265 270

Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
275 280 285 290

Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
295 300 305

Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
310 315 320

Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
325 330 335

Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
340 345 350

Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
355 360 365 370

Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
375 380 385

Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
390 395

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1368 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1368
 - (D) OTHER INFORMATION:/STANDARD NAME="60A"

- (x) PUBLICATION INFORMATION:
 - (A) AUTHORS: WHARTON, KRISTI A.; THOMSEN, GERALD H.; GELBERT, WILLIAM M.
 - (B) TITLE: DROSOPHILA 60A GENE...
 - (C) JOURNAL: PROC. NAT'L ACAD. SCI. USA
 - (D) VOLUME: 88
 - (E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 1368
 - (F) PAGES: 9214-9218
 - (G) DATE: OCT - 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC	48
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	
1 5 10 15	
CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG	96
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	
20 25 30	
GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC	144
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	
35 40 45	
CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC AAG CTG GAC GTC	192
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	
50 55 60	
TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC	240
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	
65 70 75 80	
CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG	288
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	
85 90 95	
CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG	336
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	
100 105 110	

GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala 115 120 125	384
GAC CTC GAG GAG GAT GAG GGC GAG CAG AAC TTC ATC ACC GAC Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp 130 135 140	432
CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu 145 150 155 160	480
AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg 165 170 175	528
CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asn Tyr Leu Val 180 185 190	576
ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu 195 200 205	624
ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly 210 215 220	672
ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr 225 230 235 240	720
GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His 245 250 255	768
GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala 260 265 270	816
CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly 275 280 285	864
CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly 290 295 300	912
TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GGC CAC AGC AGC CAC Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His 305 310 315 320	960

- 126 -

CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser 325 330 335	1008
G TG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg 340 345 350	1056
AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp 355 360 365	1104
CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser 370 375 380	1152
GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His 385 390 395 400	1200
GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro 405 410 415	1248
AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr 420 425 430	1296
CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile 435 440 445	1344
GTG AAA TCC TGC GGG TGC CAT TGA Val Lys Ser Cys Gly Cys His 450 455	1368

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 455 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	1	5	10	15
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	20	25	30	

Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45

Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80

Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110

Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125

Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160

Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175

Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190

Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205

Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225 230 235 240

Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
245 250 255

Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
260 265 270

His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
275 280 285

Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
290 295 300

Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305 310 315 320

His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
325 330 335

Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
340 345 350

Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
355 360 365

His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
370 375 380

Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
385 390 395 400

Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
405 410 415

Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
420 425 430

His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
435 440 445

Val Lys Ser Cys Gly Cys His
450 455

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo Sapiens
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /note="BMP3"

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 104 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1 5 10 15

Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Try Cys Ser Gly
20 25 30

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
35 40 45

Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
50 55 60

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65 70 75 80

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85 90 95

Thr Val Glu Ser Cys Ala Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1 5 10 15

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85 90 95

Arg Ser Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /note= "BMP6"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

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Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95

Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY
SELECTED FROM THE RESIDUES OCCURRING AT THE
CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE
OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 5,6,7 and 8 or
16,18,20 and 22.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95

Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 5
- (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu	Xaa	Xaa	Xaa	Phe				
1				5				
Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa
					10			
Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Ala	
		15			20			
Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa	
		25			30			
Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
					35			
Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa	
			40			45		
Xaa								
				50				
Xaa	Cys							
		55			60			

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 6
- (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
1 5 10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
20 25

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
30 35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
45 50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Xaa Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1238 base pairs, 372 amino acids
 - (B) TYPE: nucleic acid, amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) ORIGINAL SOURCE:
 - (A) ORGANISM: human
 - (F) TISSUE TYPE: BRAIN
- (iv) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:
 - (D) OTHER INFORMATION:
*/product= "GDF-1"
/note= "GDF-1 CDNA"*

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Lee, Se-Jin
(B) TITLE: Expression of Growth/Differentiation Factor 1
(C) JOURNAL: Proc. Nat'l Acad. Sci.
(D) VOLUME: 88
(E) RELEVANT RESIDUES: 1-1238
(F) PAGES: 4250-4254
(G) DATE: May-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGGGACACCCG GCCCCGCCCT CAGCCCCTG GTCCCCGGCC GCCGCGGACC CTGCGCACTC	60
TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC GGC Met Pro Pro Pro Gln Gln Gly Pro Cys Gly	113
1 5 10	
CAC CAC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC His His Leu Leu Leu Leu Ala Leu Leu Pro Ser Leu Pro	158
15 20 25	
CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu	203
30 35 40	
CAG GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu	248
45 50 55	
CGG CCG GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC Arg Pro Val Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp	293
60 65 70	
CCC CAG GAG ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val	338
75 80 85	
ACC CTG CAA CCG TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn	383
90 95 100	
ATC GTG CGC CAC ATC CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser	428
105 110 115	
GAG CCT GTC TCG GCC GCG GGG CAT TGC CCT GAG TGG ACA GTC GTC Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val	473
120 125 130	
TTC GAC CTG TCG GCT GTG GAA CCC GCT GAG CGC CCG AGC CGG GCC Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala	518
135 140 145	

CGC CTG GAG CTG CGT TTC GCG GCG GCG GCG GCA GCC CCG GAG Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu 150 155 160	563
GGC GGC TGG GAG CTG AGC GTG GCG CAA GCG GGC CAG GGC GCG GGC Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly 165 170 175	608
GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG TTG GTG CCC GCC CTG Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu 180 185 190	653
GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC GCT TGG GCT CGC Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg 195 200 205	698
AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG GCG CTA CGC Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg 210 215 220	743
CCC CGG GCC CCT GCC TGC GCG CGC CTG GCC GAG GCC TCG CTG Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu 225 230 235	788
CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC CGG Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg 240 245 250	833
CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Pro Gly Gly 255 260 265	878
GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC CAG GTG GGC Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly 270 275 280	923
TGG CAC CGC TGG GTC ATC GCG CCG CGC CCC TTC CTG GCC AAC TAC Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr 285 290 295	968
TGC CAG GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly 300 305 310	1013
GGG CCG CCG GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His 315 320 325	1058
GCG GCC GCC CCG GGA GCC GCG GAC CTG CCC TGC TGC GTG CCC GCG Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala 330 335 340	1103

CGC CTG TCG CCC ATC TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC 1148
 Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
 345 350 355

GTG GTG CTG CGG CAG TAT GAG GAC ATG GTG GTG GAC GAG TGC GGC 1193
 Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
 360 365 370

TGC CGC TAACCCGGGG CGGGCAGGGA CCCGGGCCA ACAATAATG CCGCGTGG 1238
Cys Arg
372

(34) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 372 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (iii) HYPOTHETICAL: NO
 - (iv) ANTI-SENSE: NO
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: human
 - (F) TISSUE TYPE: BRAIN
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:
 - (D) OTHER INFORMATION: /function= /product= "GDF-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
1 5 10

His His Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
15 20 25

Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
30 35 40

Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
45 50 55

Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp
60 65 70

Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val
75 80 85

Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn
90 95 100

Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser
105 110 115

Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val
120 125 130

Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala
135 140 145

Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
150 155 160

Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly
165 170 175

Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu
180 185 190

Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg
195 200 205

Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg
210 215 220

Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu
225 230 235

Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg
240 245 250

Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Pro Gly Gly
255 260 265

Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
270 275 280

Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr
285 290 295

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
300 305 310

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
315 320 325

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Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
330 335 340

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
345 350 355

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
360 365 370

Cys Arg
372

What is claimed is:

1. A therapeutic treatment method for preventing loss of bone mass or increasing bone mass in an individual, the method comprising:

administering to the individual a therapeutically effective morphogen in an amount and for a time sufficient to prevent loss of or to increase bone mass in said individual.

2. A therapeutic treatment method for preventing loss of bone mass or for increasing bone mass in an individual, comprising:

administering an agent that stimulates in vivo the effective concentration of a naturally occurring morphogen in said individual sufficient to prevent loss of or to increase bone mass in said individual.

3. The method of claim 1 or 2 wherein said loss of bone mass results from a metabolic bone disease.

4. The method of claim 3 wherein said metabolic bone disease comprises osteoporosis or osteomalacia.

5. The method of claim 1 or 2 wherein said loss of bone mass results from an imbalance in bone resorption or bone formation.

6. The method of claim 1 or 2 wherein said loss of bone mass results from an imbalance of calcium or phosphate metabolism.
7. The method of claim 1 or 2 wherein said loss of bone mass results from a vitamin D imbalance in the individual.
8. The method of claim 1 or 2 wherein said loss of bone mass is nutritionally or hormonally induced.
9. The method of claim 4 wherein said osteoporosis is postmenopausal or senile osteoporosis.
10. The method of claim 1 or 2 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).
11. The method of claim 10 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).
12. The method of claim 1 or 2 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

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13. The method of claim 12 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
14. The method of claim 1 or 2 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
15. The method of claim 1 or 2 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).
16. The method of claim 1 or 2 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).
17. A method for repairing defects in bone tissue microstructure resulting from a metabolic bone disease, the method comprising:
administering to an individual a therapeutically effective morphogen in an amount and for a time sufficient to repair defects in said microstructure.
18. A method for repairing defects in bone tissue microstructure resulting from a metabolic bone disease, the method comprising:
administering an agent capable of stimulating in vivo the effective concentration of a naturally occurring morphogen sufficient to repair said defects.

19. The method of claim 17 or 18 wherein said metabolic bone disease comprises osteoporosis or osteomalacia.

20. A method for protecting an individual at risk for loss of bone mass, the method comprising:

providing to the individual a therapeutically effective morphogen in an amount and for a time sufficient to protect said individual from loss of bone mass.

21. A method for protecting an individual at risk for loss of bone mass, the method comprising:

providing to the individual an agent that stimulates in vivo a therapeutically effective concentration of a naturally occurring morphogen sufficient to protect said individual from loss of bone mass.

22. The method of claim 20 or 21 wherein said individual is a postmenopausal female or is undergoing dialysis.

23. The method of claim 20 or 21 wherein said individual is at risk for loss of bone mass as a result of senile osteoporosis.

24. The method of claim 17, 18, 20 or 21 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).

25. The method of claim 24 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).
26. The method of claim 17, 18, 20 or 21 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
27. The method of claim 26 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
28. The method of claim 17, 18, 20 or 21 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
29. The method of claim 17, 18, 20 or 21 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).
30. The method of claim 17, 18, 20 or 21 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).

31. The method of claim 1, 2, 17, 18, 20 or 21, wherein said morphogen or said morphogen-stimulating agent is provided to the individual by oral administration.
32. The method of claim 1, 2, 17, 18, 20 or 21, wherein said morphogen or morphogen-stimulating agent is provided to the individual by parenteral administration.
33. The method of claim 1, 17, or 20 wherein said morphogen is provided to said individual in association with a molecule capable of enhancing the solubility of said morphogen.
34. The method of claim 33 wherein said molecule comprises casein or part or all of the pro domain of a morphogen.
35. The method of claim 34 wherein said pro domain comprises part or all of the sequence described by residues 30 to 292 of Seq. ID No. 16.
36. The method of claim 1, 17, or 20 wherein said morphogen is provided to the individual in association with a molecule capable of targeting said morphogen to bone tissue.
37. The method of claim 36 wherein said targeting molecule comprises tetracycline, diphosphonates, or an antibody that binds specifically to a molecule on the surface of bone tissue cells.

38. The method of claim 2, 18 or 21 wherein said morphogen-stimulating agent is provided to the individual in association with a molecule capable of targeting said agent to morphogen-producing or morphogen-secreting tissue.
39. A morphogen useful in the manufacture of a pharmaceutical for use in a treatment for preventing loss of bone mass or for increasing bone mass in an individual.
40. A therapeutic agent useful in the manufacture of a pharmaceutical for use in a treatment for preventing loss of bone mass or for increasing bone mass in an individual, the agent being capable of stimulating in vivo an effective concentration of a naturally occurring morphogen, sufficient to prevent loss of or to increase bone mass in the individual.
41. The pharmaceutical of claim 39 or 40 for use in a treatment to prevent bone loss due to a metabolic bone disease.
42. The pharmaceutical of claim 39 or 40 for use in a treatment to prevent bone loss due to an imbalance in bone resorption or bone formation.
43. The pharmaceutical of claim 41 for use in treating osteomalacia or osteoporosis.
44. A morphogen useful in the manufacture of a pharmaceutical for use in a treatment for protecting an individual at risk for loss of bone mass.

45. A therapeutic agent useful in the manufacture of a pharmaceutical for use in a treatment for protecting an individual at risk for loss of bone mass, the agent being capable of stimulating in vivo an effective concentration of a naturally occurring morphogen sufficient to protect said individual from loss of bone mass.
46. The pharmaceutical of claim 44 or 45 for use in a treatment to protect an individual at risk for loss of bone mass as a result of senile osteoporosis.
47. The pharmaceutical of claim 44 or 45 for use in a treatment of an individual at risk for loss of bone mass as a result of undergoing dialysis.
48. The pharmaceutical of claim 39, 40, 44 or 45 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with a sequence selected from the group consisting of OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).
49. The pharmaceutical of claim 48 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), BMP3(fx), BMP5(fx), BMP6(fx) and 60A(fx).
50. The pharmaceutical of claim 39, 40, 44 or 45 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

51. The pharmaceutical of claim 50 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
52. The pharmaceutical of claim 39, 40, 44 or 45 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
53. The pharmaceutical of claim 39, 40, 44 or 45 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).
54. The pharmaceutical of claim 39, 40, 44 or 45 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).
55. A composition useful in a treatment to prevent loss of bone mass or to increase bone mass in an individual, the composition comprising a morphogen or morphogen-stimulating agent in association with a bone tissue targeting molecule.
56. The composition of claim 55 wherein said bone tissue targeting molecule comprises tetracycline, a diphosphonate, or an antibody or antibody fragment that binds specifically to a molecule on the surface of bone tissue cells.

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57. A composition useful in a treatment to prevent loss of bone mass or to increase bone mass in an individual, the composition comprising a morphogen or morphogen stimulating agent in association with a cofactor.
58. The composition of claim 57 wherein said cofactor is selected from the group consisting of vitamin D₃, calcitonin, a prostaglandin, parathyroid hormone, dexamethasone, estrogen and IGF.
59. The composition of claim 55 wherein said composition is provided to said individual in association with a molecule capable of enhancing the solubility of said morphogen.

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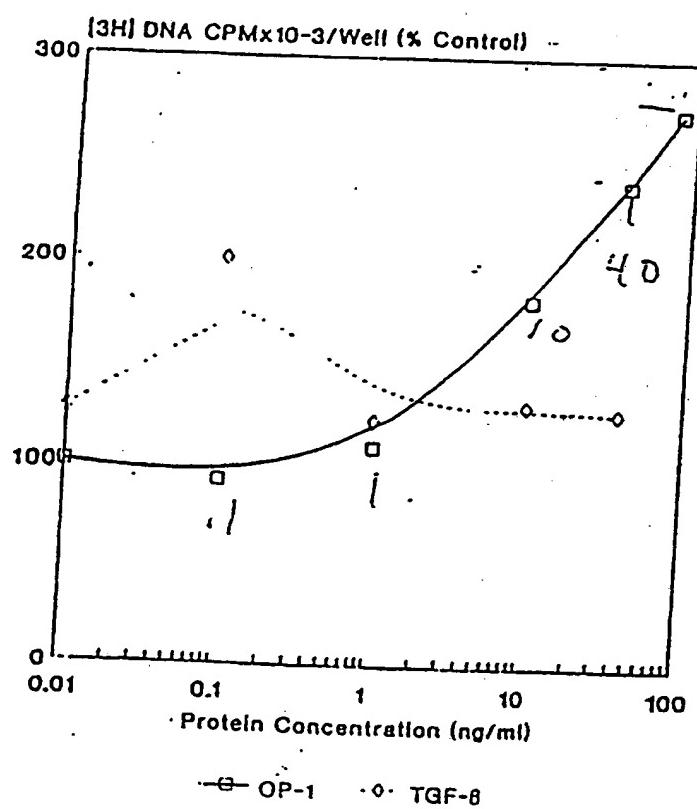


Figure 1

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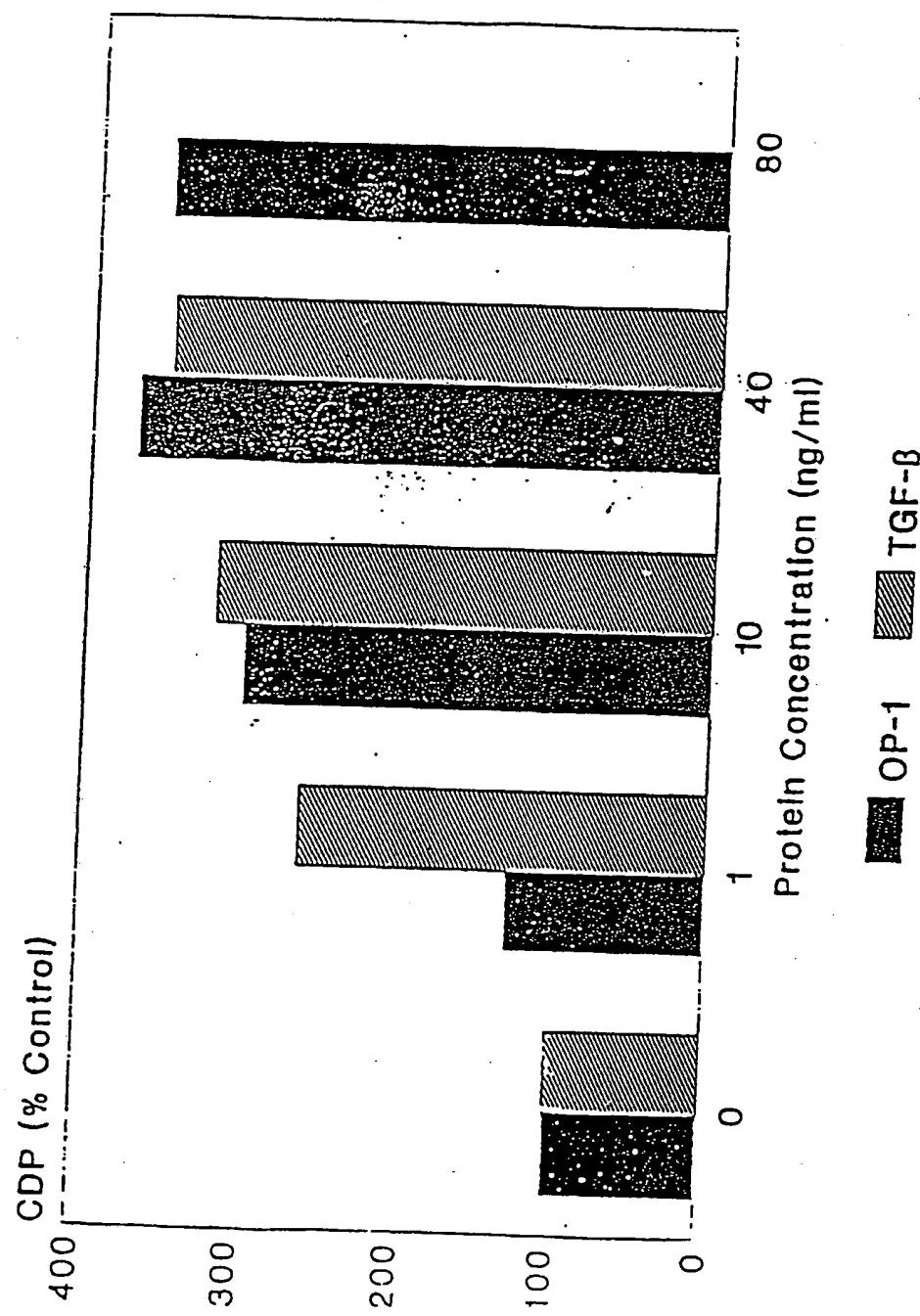


Figure 2

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PROTEIN CONCENTRATION (ng/ml)	cAMP (pmole/well)	
	-PTH	+PTH
Background	1.30	2.20
OP-1	1.0 10.0 40.0	1.25 1.30 1.25
TGF-β	0.1 1.0 5.0	0.95 0.83 0.68

Figure 3

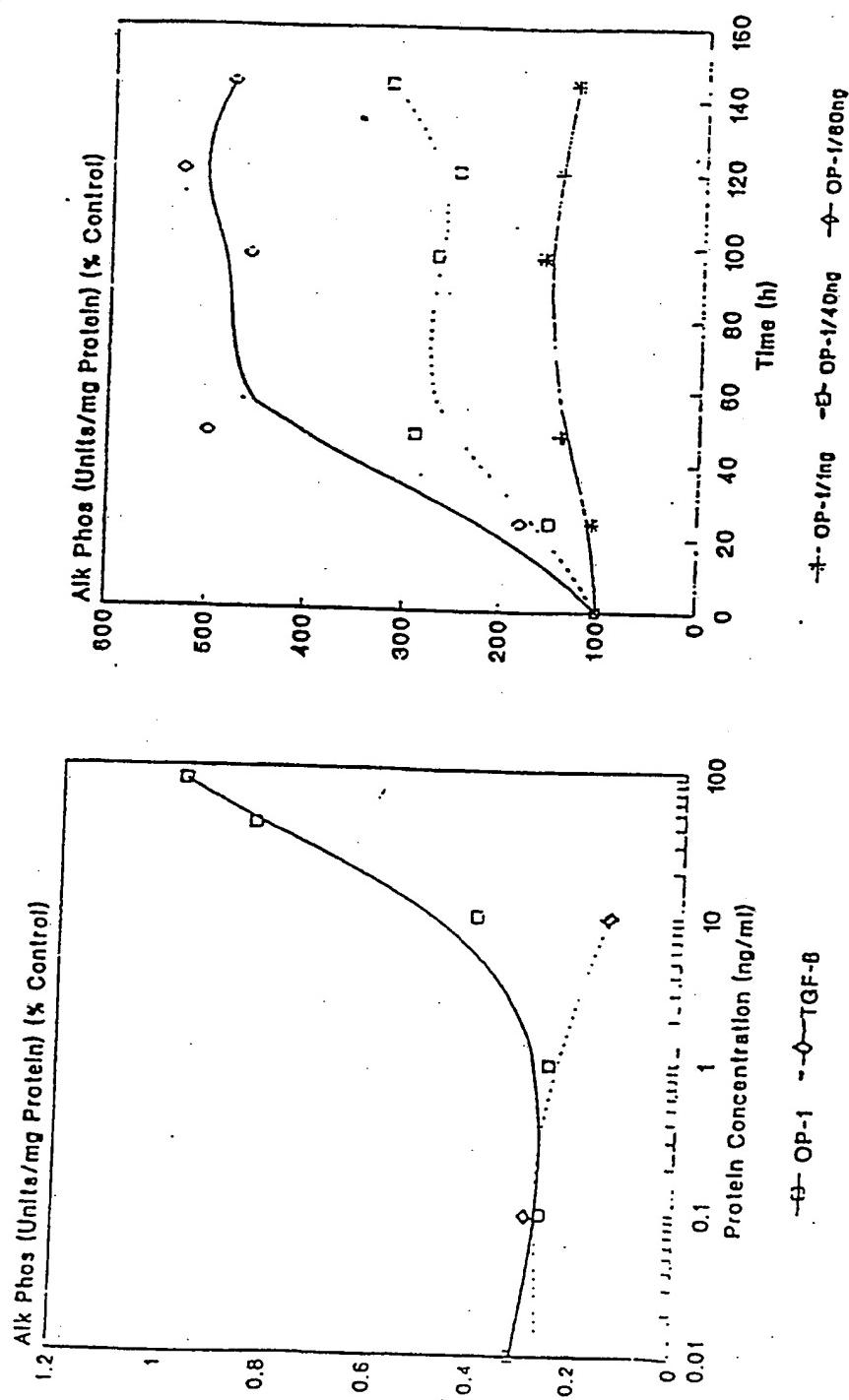


Figure 4

Figure 5

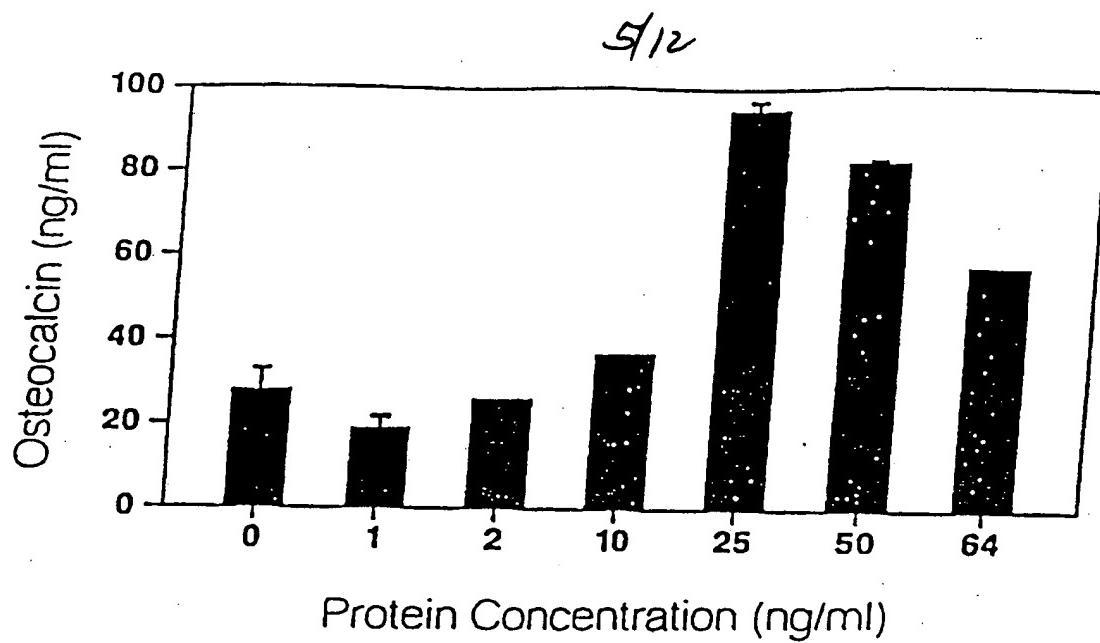


Figure 6A

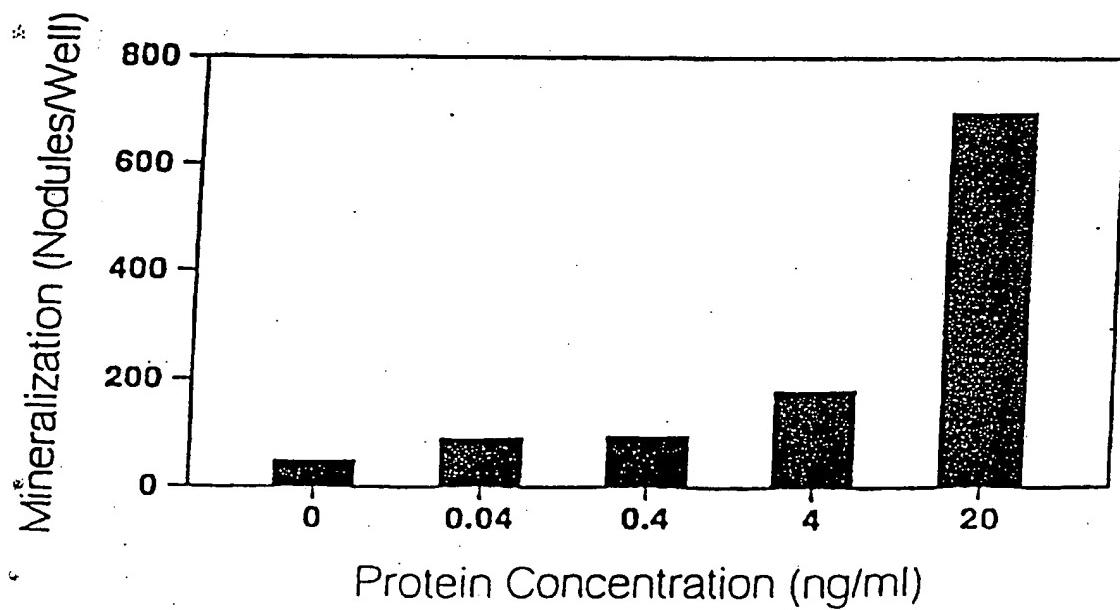
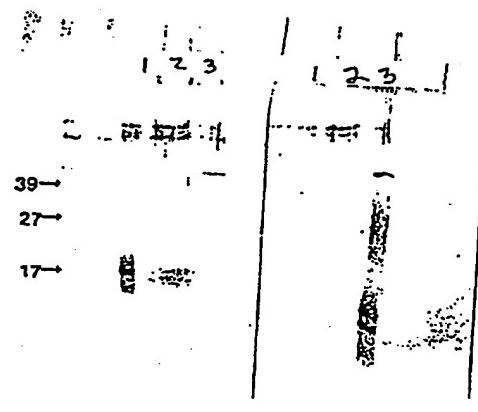


Figure 6B

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A

B

FIG 7

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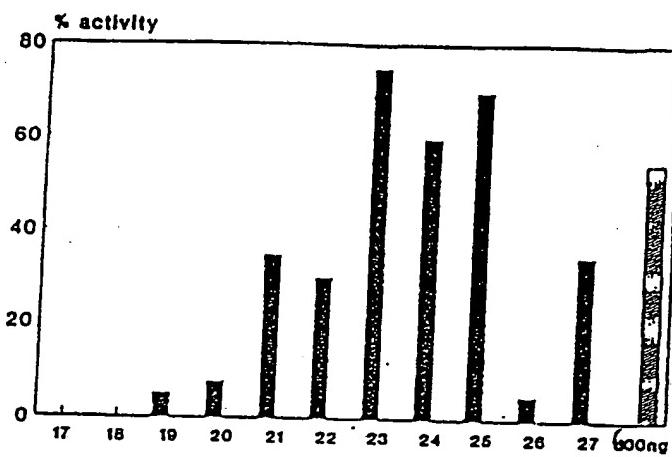


FIG 8A

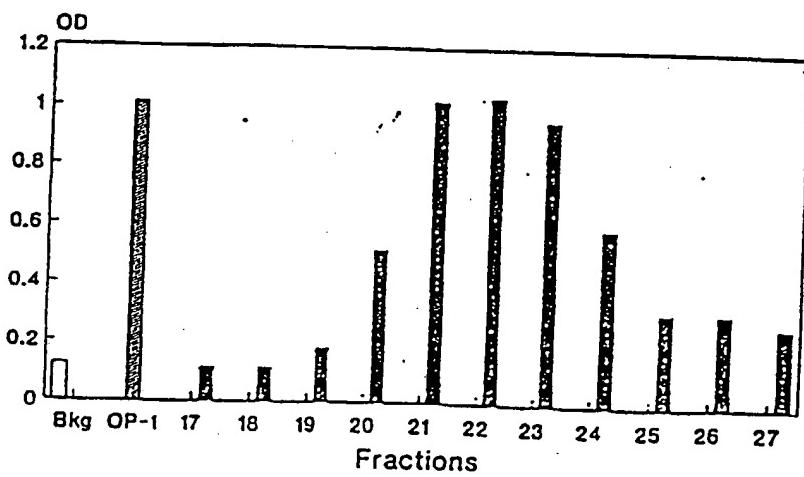
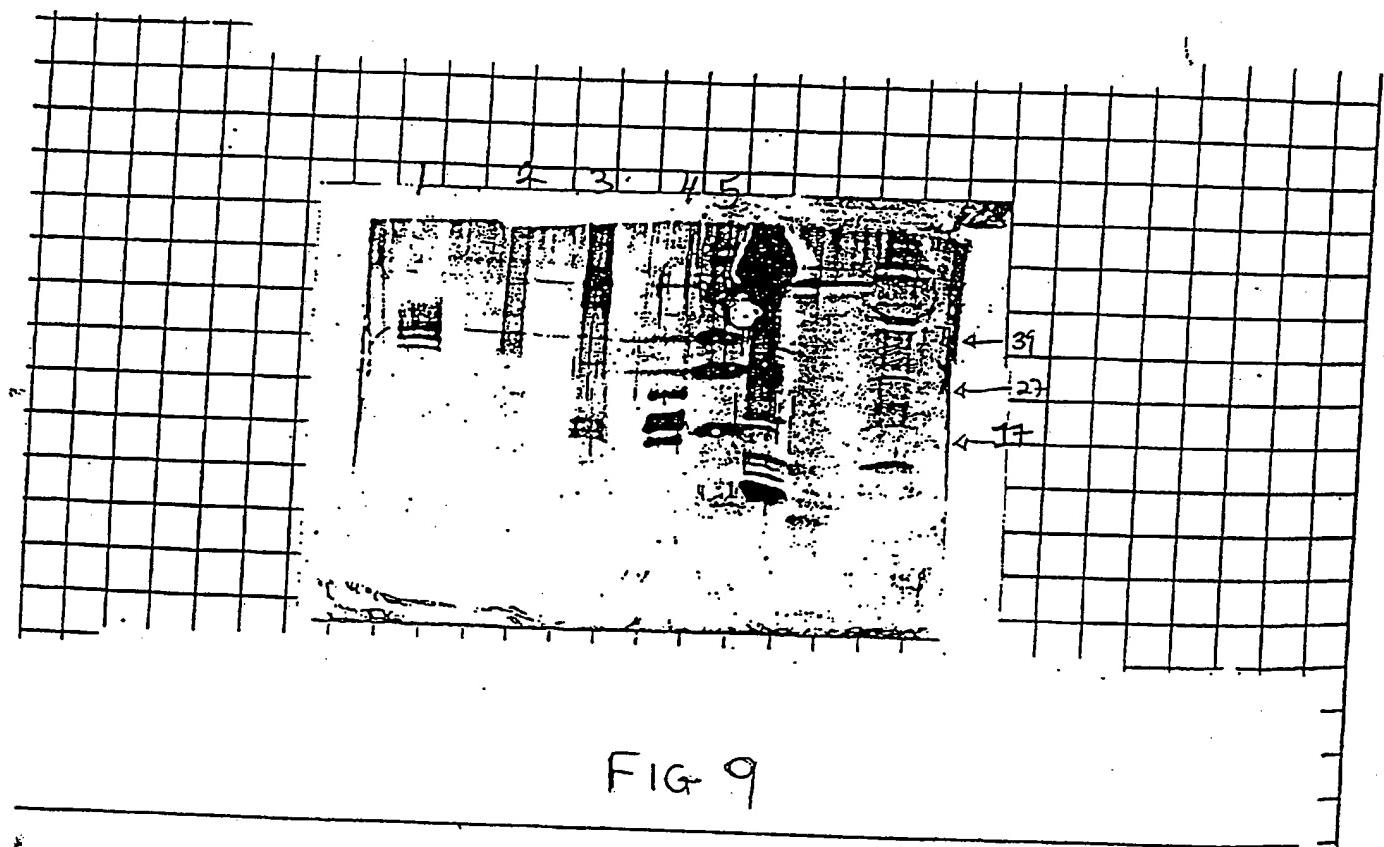


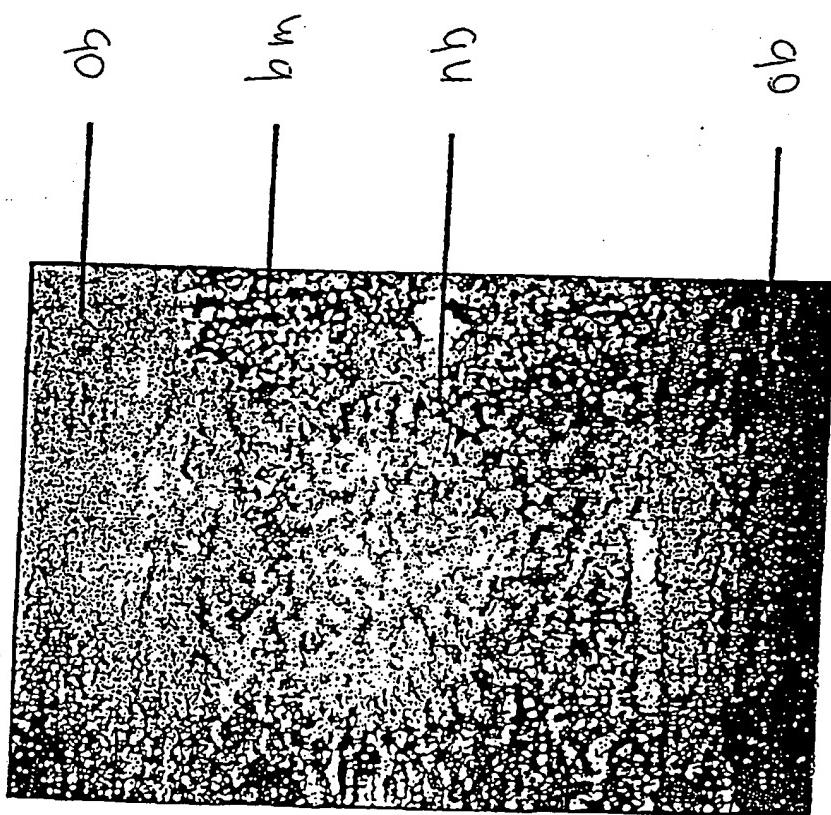
FIG 8B

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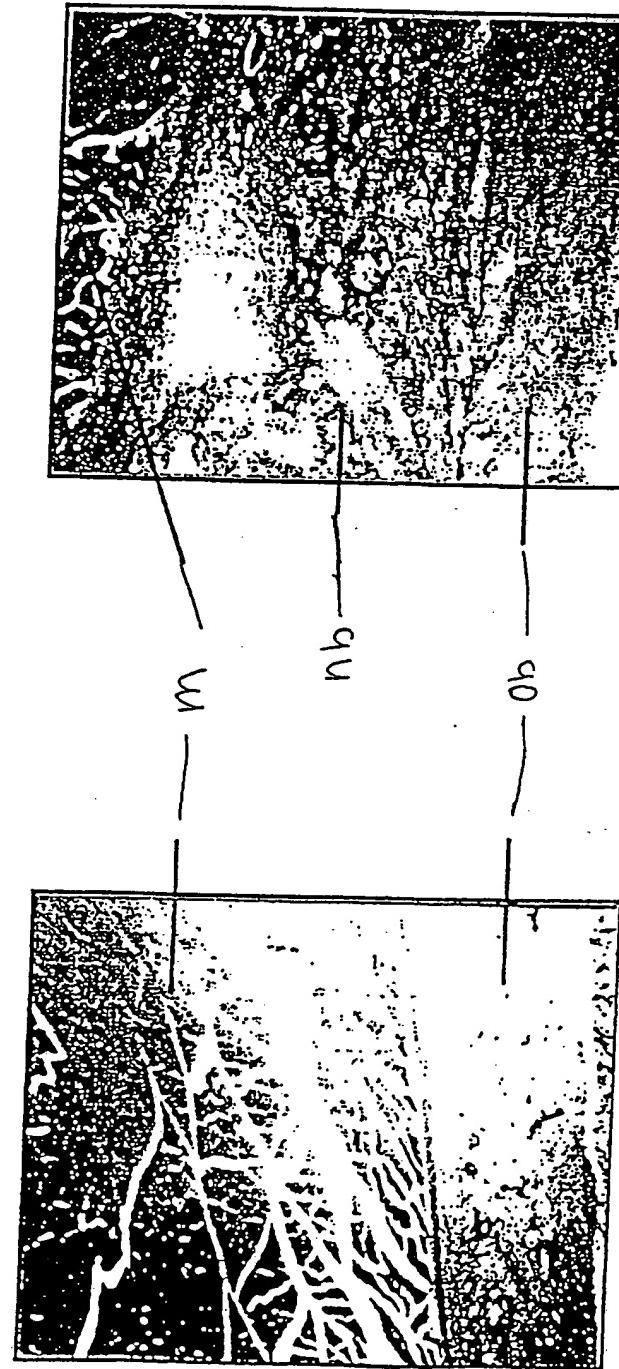
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Fig 10A



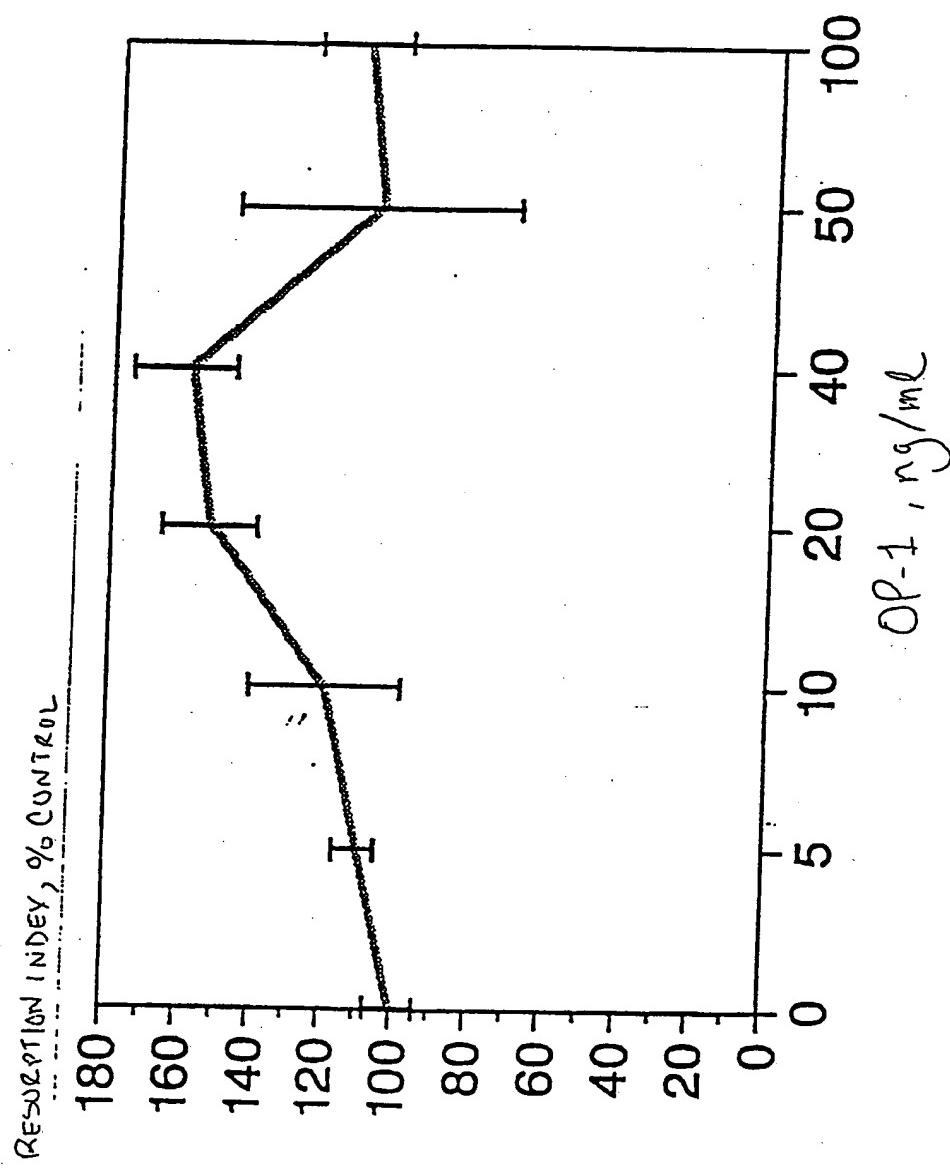
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Fig 10B

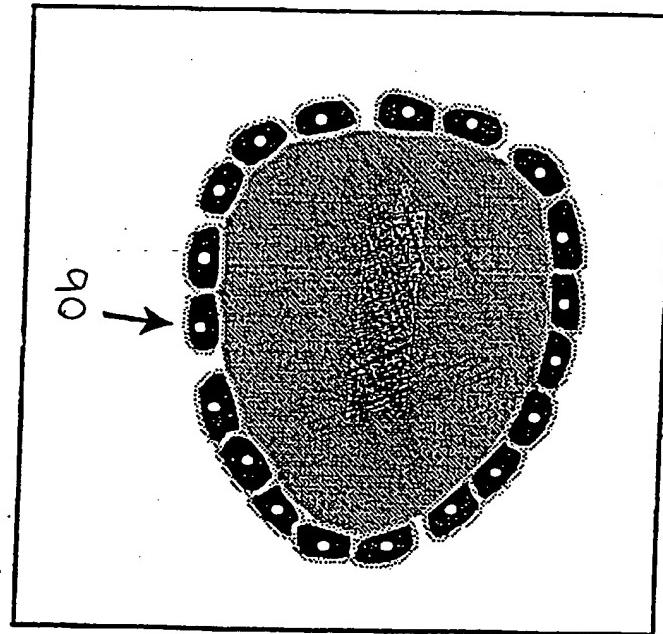


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Fig. 11

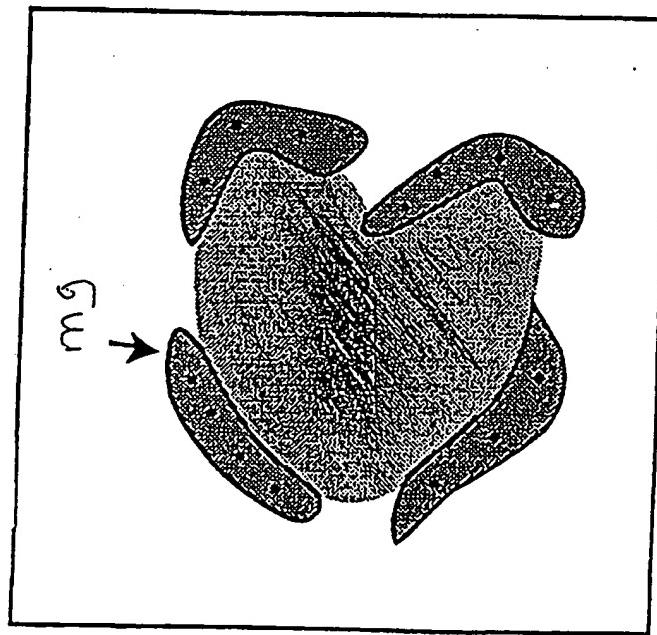


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B

FIG. 12



A

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